

Behavioural Mechanisms behind Aggregation in a Tritrophic Perspective

Jörg Stephan

Faculty of Forest Science

Department of Ecology

Uppsala

Doctoral Thesis

Swedish University of Agricultural Sciences

Uppsala 2016

Acta Universitatis agriculturae Sueciae

2016:34

Cover: Word cloud generated with the web-application Tagxedo based on the text of all papers/manuscripts of the thesis. Image of me modified with ImageJ.
(photo: J. Stephan)

ISSN 1652-6880

ISBN (print version) 978-91-576-8570-4

ISBN (electronic version) 978-91-576-8571-1

© 2016 Jörg Stephan, Uppsala

Print: SLU Service/Repro, Uppsala 2016

Behavioural Mechanisms behind Aggregation in a Tritrophic Perspective

Abstract

Gregarious organisms need to handle the trade-off between positive effects of group living and increasing food competition. The gregarious *Phratora vulgatissima*, a specialist leaf beetle on willow in Europe and Asia, frequently reaches outbreak densities in natural stands and short rotation coppices. Outbreaks threaten the yield and plantations therefore rely on omnivorous predators as biocontrol agents, like *Anthocoris nemorum* and *Orthotylus marginalis*. I aimed to elucidate behavioural mechanisms of the beetle and the predators to understand how and why species aggregate. The beetle's aggregation behaviour was studied by looking at key reproductive traits like oviposition rate, clutch size, and oviposition site choice on willow shoots. Both predators were characterized further by examining where on the vertical shoot they preferentially hunt for the beetle's eggs and how the quality of alternative food (different plant genotypes) alters their effects on the beetle. I so revealed how the reproduction of the beetle is modulated by lateral (conspecific density), bottom-up (plant genotypes), and top-down (omnivorous predators) effects. To lower exploitative competition among larvae females increase the distances between clutches on a plant and lower their oviposition rate if too many, or too few (too few confirmations of own decision) conspecifics visited a shoot. Observed bottom-up effects include lowering clutch size and number of eggs on a shoot with plant genotype unsuitability, initially selecting large leaves for oviposition/feeding, and increasing clutch distances due to larger leaf area of a willow genotype. Three top-down effects in form of predator avoidance behaviours were observed. Females lowered median clutch size and oviposition rate leading to fewer eggs in presence of the predators/their combination. Females also preferentially feed in the shoot canopy but oviposit in the lower part. In combination with the observation that both predators show contrasting vertical preferences on the shoot I argue that females try to avoid the area where the predators are consuming more eggs. In general I contributed to the ecological concept of habitat domain, attack-abatement, clutch size, cognitive maps, and nonconsumptive effects. I hope the results facilitate our understanding of insect outbreaks and species aggregation, possibly leading to better control of those in economic relevant systems.

Keywords: clutch size, cognitive map, kin recognition, non-consumptive effects, oviposition rate, neutral interference, selfish herd, habitat quality, dilution, omnivore

Author's address: Jörg Stephan, SLU, Department of Ecology,
P.O. Box 7044, 750 07 Uppsala, Sweden
E-mail: jorg.stephan@slu.se

Dedication

To my wonderful wife and daughter

Contents

List of Publications	7
Abbreviations	9
1 Introduction	11
1.1 Oviposition and aggregation in response to intraspecific competition	11
1.2 Oviposition and aggregation in response to host plants and predators	13
1.2.1 Not laying eggs as a nonconsumptive effect	13
1.2.2 Changing the oviposition site on an individual willow shoot	14
2 Thesis aims	17
3 Study system	19
3.1 The leaf beetle	19
3.2 Willow as host plant	19
3.3 The omnivorous predators	20
4 Methods	21
4.1 Beetle oviposition in presence of conspecific eggs	21
4.2 Oviposition in response to host plants and predators	22
4.2.1 Clutch size and number of eggs laid on a shoot	22
4.2.2 Vertical position of eggs on the shoot	23
5 Results and Discussion	25
5.1 Beetle oviposition in presence of conspecific eggs	25
5.2 Oviposition in response to host plants and predators	26
5.2.1 Clutch size and number of eggs laid on a shoot	26
5.2.2 Vertical position of eggs on the shoot	29
6 Conclusion	33
References	35
Acknowledgements	43

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I Stephan JG, Stenberg JA, Björkman C (2015). How far away is the next basket of eggs? Spatial memory and perceived cues shape aggregation patterns in a leaf beetle. *Ecology* 96(4), 908-914.
- II Stephan JG, Low M, Stenberg JA, Björkman C (2016). Habitat quality and predator hunting mode interactively affect attack-abatement patterns of predation risk. *Ecology* (submitted).
- III Stephan JG, Stenberg JA, Björkman C. Consumptive and nonconsumptive effect ratio depends on local resource quality – a story about host plants, leaf beetles, and omnivorous predators (manuscript).
- IV Stephan JG, Stenberg JA, Björkman C. Moving up and down the shoot: how ovipositing leaf beetles avoid habitat domains of different predators (manuscript).

Paper I is reproduced with the permission of the publisher.

The contribution of Jörg Stephan to the papers included in this thesis was as follows:

- I Developed research questions and design, performed data collection, did the statistical analyses and wrote the paper with assistance from Johan Stenberg and Christer Björkman.
- II Developed research questions and wrote the paper together with Matt Low with assistance from Johan Stenberg and Christer Björkman.
- III Developed research questions, performed data collection together with Johan Stenberg, did the statistical analyses and wrote the paper with assistance from Johan Stenberg and Christer Björkman.
- IV Developed research questions, performed data collection together with Johan Stenberg, did the statistical analyses and wrote the paper with assistance from Johan Stenberg and Christer Björkman.

Abbreviations

1 AN + 1 OM	Predator treatment with one <i>Anthocoris nemorum</i> and one <i>Orthotylus marginalis</i> caged on individual willow shoots
2 AN	Predator treatment with two <i>A. nemorum</i> caged on individual willow shoots
2 OM	Predator treatment with two <i>O. marginalis</i> caged on individual willow shoots
4 OM	Predator treatment with four <i>O. marginalis</i> caged on individual willow shoots

1 Introduction

The aim of this thesis was to shed light on the questions how and why species aggregate. These questions were approached with controlled lab experiments utilizing a leaf beetle, different host plant species/genotypes and two omnivorous predators. I tried to include three levels within the trophic system in investigating how predators (top-down the food chain), how the host plant (bottom-up), and lateral (on the same trophic level) effects shape the beetles' behaviour. I aim to understand if and how the reproductive behaviour of the herbivore is modulated by these mechanism on all these trophic levels as this early phase during beetle life time is critical for the fitness and the population dynamics of the species with the potential to get insights on the occurrence of insect outbreaks.

1.1 Oviposition and aggregation in response to intraspecific competition

Due to exploitative competition (Mitchell, 1975) and increased risk of predation while searching for alternative feeding sites (Matsumoto, 1990) survival of insect larvae normally decreases with increasing egg numbers in an aggregation. On the other hand, larval aggregation may improve thermoregulation (Joos *et al.*, 1988), increase predator protection (Denno & Benrey, 1997; Hunter, 2000), and help to overcome plant defences (Clark & Faeth, 1997). Larvae are aggregated because mothers chose where to place the eggs on a plant (Whitham, 1978; Silva & Furlong, 2012) and within the landscape (Heisswolf *et al.*, 2006). This oviposition site choice can be modulated by e.g. vegetation structure (Meiners & Obermaier, 2004), predators/parasitoids (Higashiura, 1989), and the availability and quality of host plants (Obermaier & Zwölfer, 1999). Information about presence of conspecific eggs might also influence the oviposition choice, with some

species preferring egg-free hosts (Vasconcellos-Neto & Monteiro, 1993), while other prefer the presence of conspecific eggs (Navasero & Ramaswamy, 1993; Raitanen *et al.*, 2013). Some species may even balance parasitism threats and larval feeding competition (Meiners *et al.*, 2005). Here we investigated how the leaf beetle *P. vulgatissima* (Coleoptera: Chrysomelidae) is distributing its eggs on individual host plants with special focus on how they respond to the presence of conspecific egg clutches.

On the individual plant the second decision by the female is how many eggs to oviposit in a clutch, which can depend on resource quality (Bergström *et al.*, 2006), resource size (Godfray, 1986), and predation/parasitism (Subinprasert & Svensson, 1988; Siemsen & Johnson, 1992). Similar predation and competition as for progeny from a single clutch could arise from siblings/non-siblings from different clutches that are on the same shared plant. Comparable to the optimal clutch size that produces the highest progeny survival (Lack's clutch-size hypothesis; Lack, 1947; Godfray *et al.*, 1991), one could imagine an optimal distance between clutches as an additional behavioural component to increase egg survival. It has been shown that the dispersal distance in spider mites populations can be driven by relatedness (Bitume *et al.*, 2013) and the spatial distribution of adults on a plant increases with inbreeding (Le Goff *et al.*, 2009). Some insects are also able to recognize conspecific eggs (Loeb *et al.*, 2000; Zink, 2003). However, how this affects distances between clutches has been unknown and is addressed in this thesis.

Insects may not only perceive eggs directly, but also indirectly via cues like sex pheromones, aggregation pheromones, faeces, and larval defence secretions (Fernandez and Hilker 2007), as well as induced plant volatiles due to feeding and oviposition (Dicke & Baldwin, 2010). At the same time insects are able to learn and memorize (Dukas, 2008; Wright & Schiestl, 2009) including spatial memory (Srinivasan, 2010; Collett *et al.*, 2013). However, these studies are performed on bees, wasps, and ants, while studies on beetles have focused on conditioning (Held *et al.*, 2001) and how larval experience influences adult behaviour (Rausher, 1983). Here we investigated for the first time the spatial memory of a leaf beetle. The existence of such cognitive capabilities should help to better understand the behaviour of this beetle on individual plants and this knowledge will aid to understand the population dynamics in this species.

1.2 Oviposition and aggregation in response to host plants and predators

Besides the direct consumptive effect on their prey, predators exhibit another indirect top-down effect. This nonconsumptive effect is associated with changes in prey traits due to scaring the prey and altering its behaviour. Nonconsumptive effects can have far-reaching impacts on trophic cascades (Beckerman *et al.*, 1997; Trussell *et al.*, 2003), ecosystem functions (Schmitz *et al.*, 2008; Matassa & Trussell, 2011), and often equals or exceeds the effects of direct consumption (Schmitz *et al.*, 2004; Preisser *et al.*, 2005). Within the system consisting of two predators, *Anthocoris nemorum* (Heteroptera: Anthocoridae) and *Orthotylus marginalis* (Heteroptera: Miridae), the leaf beetle *P. vulgatissima* and different *Salix* spp. genotypes (Malpighiales: Salicaceae) we addressed two nonconsumptive effects. First, we focused on the effect of predator presence on the clutch size and the oviposition rate of the leaf beetle. Second, the oviposition site selection due to predator presence was investigated. More specifically, we looked at the distribution of herbivore eggs and hunting efforts of predators on the vertical axis of willow shoots and if the beetles changed their oviposition site preferences due to predators.

1.2.1 Not laying eggs as a nonconsumptive effect

The presence of predators can for example generate physiological stress resulting in energetic costs cascading an negative impact on reproductive output (Nelson, 2007). This should represent the strongest nonconsumptive effect because it lowers fitness of the prey. This effect is for example exerted via higher conspicuousness of males attracting females (Uzendoski *et al.*, 1993), mating interruption (Travers & Sih, 1991), or changes in prey behaviour that result in lower weight gain or poorer provision of progeny (Harfenist & Ydenberg, 1995).

Besides nutritional value the herbivores behaviour can be affected by the plant quality expressed as different combinations of defence traits (Agrawal, 2007; Schaller, 2008; Karban, 2011) in form of structural features like trichomes (Mulatu *et al.*, 2006) and chemical features like volatiles (Degen *et al.*, 2004). These difference not only exist among species but also among plant genotypes (Kaplan & Thaler, 2010; Stenberg *et al.*, 2011a) affecting herbivore performance (Kaplan & Thaler, 2010), fitness (Lehrman *et al.*, 2012) and community composition (Schmitz *et al.*, 2008; Wimp *et al.*, 2010).

Plant genotype also affects higher trophic levels (Underwood & Rausher, 2000; Bailey *et al.*, 2006; Tack *et al.*, 2010), and efforts have been made to connect nonconsumptive effects of predators to the plant genotype the interaction occurs on (Thaler *et al.*, 2014; Kersch-Becker & Thaler, 2015).

Even if the omnivore is not affected by structural defences, variation in plant sap quality can alter the means of satisfying nutritional needs leading to higher or lower consumption of herbivores (Lundgren *et al.*, 2009; Stenberg *et al.*, 2011b). However, no attempts have been made to tease apart the contributions of consumptive and nonconsumptive effect on fitness of an individual herbivore and the combined effects of different predators. We therefore explored if the oviposition rate is altered by host plant genotype, predator presence and how different host plants genotypes interact with this nonconsumptive effect because the omnivorous predator is also affected by host plant quality.

Whether herbivores lay fewer eggs within a certain time (lower oviposition rate) can depend on plant species richness in the habitat (Unsicker *et al.*, 2010), temperature (Tammaru *et al.*, 1996), or intraspecific exploitative competition (Hemptinne *et al.*, 1992). Oviposition rate can also be a proxy for host plant (genotype) acceptance in the *P. vulgarissima*-willow system (Lehrman *et al.*, 2012) and we used this observation to investigate how different predators and different willow genotypes modulate this nonconsumptive effect.

In addition to how many eggs are laid on an individual plant, how many eggs are laid in a certain location is important in determining egg aggregation. These egg clutches (also: batch, cluster, patch) have contact with each other and bottom-up like resource size and quality (Godfray, 1986; Pilon & Rausher, 1988; Kagata & Ohgushi, 2002) and top-down factors like predator/parasitoid attack (Subinprasert & Svensson, 1988; Siemens & Johnson, 1992) have been shown to be important in determining their size. Females would increase fitness by laying more eggs in the same clutch as it could increase the ability to overcome different plant defences (Young & Moffett, 1979; Clark & Faeth, 1997), or lower the encounter probability of predators (Paper II). On the other hand large clutches will increase exploitative competition between the hatching larvae (Mitchell, 1975) forcing them to migrate, which in turn can increase predation risk (Matsumoto, 1990). How the predator is foraging on the herbivore eggs is also determining if larger clutch sizes are advantageous. Differences in this predator hunting mode (Miller *et al.*, 2014) increased survival chance in larger clutches in cases were the predator is not immediately consuming all encountered eggs (Paper II). Therefore if the clutch size of the leaf beetle is interactively affected by top-down and bottom-up effects was investigated.

1.2.2 Changing the oviposition site on an individual willow shoot

Oviposition site choice is a key life history trait in insect ecology (Refsnider & Janzen, 2010), important at different spatial scales (Kessler & Baldwin, 2002;

Meiners & Obermaier, 2004; Silva & Furlong, 2012). Females have to balance sufficient food availability for the larvae and predation risk in their choice where to lay their eggs on individual plants (Kessler & Baldwin, 2002). The predators can differ in the danger that they present to the progeny as they exhibit different hunting modes and habitat domains (Miller *et al.*, 2014). *A. nemorum* shows a ‘run and eat’ hunting mode, while *O. marginalis* is less mobile and can be considered as a ‘find and stay’ predator (Björkman *et al.*, 2003). These contrasting modes were used to explain how the predation on leaf beetle eggs and larvae is negatively affected via intraspecific interactions in the mobile predator *A. nemorum* but not in the less mobile predator *O. marginalis* (Björkman & Liman, 2005). Hunting mode also served as explanation for the neutral interspecific interactions between the two predators (Björkman & Liman, 2005). However, different habitat domains on the vertical willow shoot axis could play an additional role in explaining intra- and interspecific interaction and were, therefore, addressed here.

More significantly, the leaf beetle may perceive some parts of the shoot less dangerous because of a small domain overlay with a predator leading to lower chances of encountering the predator. Here we investigated the habitat domains of the leaf beetle and the two predators, their overlay, and if the anticipation of predation on the beetle eggs can change the oviposition site selection by beetle females (Vonesh & Blaustein, 2010; Lee *et al.*, 2014). Investigating where on the shoot the leaf beetle is ovipositing and where important predators in the system hunt for these eggs should help to understand if predation pressure could have been involved in the evolution of the oviposition site selection of the leaf beetle. We investigated the nonconsumptive effect of changing oviposition site in a set up where the predators is actually an omnivores which may be more affected by plant quality than the herbivorous prey they consume (Eubanks & Denno, 1999). Thus, we investigated how different plant genotypes change these vertical preferences of the predators and the leaf beetle and if it also changes the responses of the leaf beetle to the respective predators and their combination.

2 Thesis aims

The aim of this thesis was to understand how and why an herbivore, the leaf beetle *P. vulgatissima*, is grouping on individual host plants. Understanding the behavioural mechanisms during oviposition by individual beetle females on individual plants should help to understand how this species is aggregating. Because population dynamics are shaped by decisions of each individual the understanding of individual behaviour ultimately contributes to the understanding of insect outbreaks. We specifically aimed:

- to investigate if leaf beetle females use spatial memory during oviposition on individual plants (Paper I)
- to connect egg predation within clutches to general predator effects on prey grouping (Paper II)
- to identify and quantify plastic behavioural responses of ovipositing leaf beetles to predators and host plants (Paper III, IV)

3 Study system

3.1 The leaf beetle

Phratora vulgatissima (Coleoptera: Chrysomelidae) [L.] adults and larvae skeletonize willow leaves (Malpighiales: Salicaceae: *Salix* spp.)[L.], and this beetle is the most common specialist herbivore of willow in Europe (Peacock & Herrick, 2000). Adults emerge in April, feed for about two weeks, mate, and subsequently lay hundreds of eggs on the undersides of leaves in clutches of 1–50 eggs. The larvae feed gregariously during the first and second instar and then solitarily on different leaves during the third instar (Kendall *et al.*, 1996) followed by pupation in the soil. Adults emerge in August, feed shortly, and find hibernation sites in reeds or under the bark of trees (Björkman & Eklund, 2006). Although the species is considered to be univoltine in Sweden it has a second generation in the Uppsala area if the first generation is completed before August (Dalin, 2011). Adults probably excrete pheromones that attract other individuals (Peacock *et al.*, 2001). Because its oviposition shows no apparent link to the survival of adults oviposition rate is a valid proxy for leaf beetle fitness (Lehrman *et al.*, 2012).

From an economical perspective this beetle is the most important insect pest in willow short rotation coppices because it can reduce the stem wood production up to 40% (Björkman *et al.*, 2000) and frequently reaches high densities (Björkman *et al.*, 2004).

3.2 Willow as host plant

The host plant of this leaf beetle is willow. Willows are grown in short-rotation coppices and have become an important system for growing renewable feedstock for bioenergy production in many countries (Keoleian & Volk, 2005; Karp & Shield, 2008).

The four *Salix* genotypes used for the experiments were chosen because they differ in chemical composition (Lehrman *et al.*, 2012) and have been used in previous experiments establishing a narrow but distinct suitability gradient for both the leaf beetle and the omnivorous predator *A. nemorum*. The suitability of these genotypes for the leaf beetle increase in the order Gudrun < Loden < 78021 < 78183 (Stenberg *et al.*, 2010). The suitability for *A. nemorum* in the absence of prey follows the reverse order. In presence of additional prey the most suitable of these genotypes for *A. nemorum* is genotype 78183 and the suitability's of the genotypes Gudrun, Loden, and 78021 are similar (Stenberg *et al.*, 2011a).

3.3 The omnivorous predators

Within the well investigated tritrophic system of the host plant willow, the leaf beetle *P. vulgatissima* and its omnivorous predators several species have been found to be important for biocontrol within willow short rotation coppices and in natural willow stands. Among them are the mirids (Heteroptera: Miridae) *Orthotylus marginalis* [Reut.] and *Closterotomus fulvumaculatus* [De Geer] (Björkman *et al.*, 2004; Dalin, 2006) and the anthocorid *Anthocoris nemorum* (Hemiptera: Anthocoridae) [L.] (Björkman *et al.*, 2004), which is also an important biocontrol agent in apple orchards (Sigsgaard 2010). *O. marginalis* is mainly predacious (Lehman 1932). Other observations suggest that it can survive on a minimal amount of animal food but has a preference for such food (Kullenberg 1944). *A. nemorum* is mostly regarded as a predator, but it also feeds on shallowly located fluids from the green parts of host plants (Lauenstein 1979).

4 Methods

In all three experiments shown here, 20-cm winter cuttings were used to grow shoots of around 60 cm height. Growing the plants and all experiments were performed in the green house (23°C, relative humidity of 80%, light regime 18 h light/6 h dark). Some days before the experiment we removed side shoots, dried leaves, and leaves not fully expanded at the top and the individual plants were placed in cylindrical transparent plastic cages (70 cm height, 30 cm diameter) with a net on top (Fig. 1). Except for the first experiment where we also used individuals from the rearing the used leaf beetles were collected in the Uppsala area, Sweden.



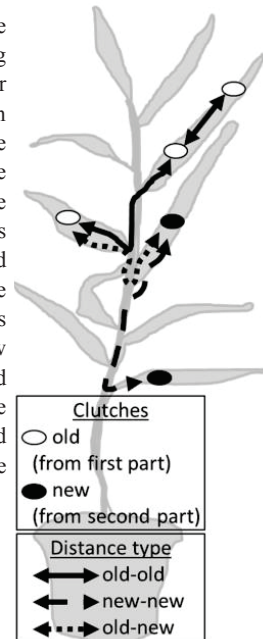
Figure 1: Caged individual plants (in the middle: uncaged plant of *Salix* genotype 78183) where leaf beetles and predators were released in.

4.1 Beetle oviposition in presence of conspecific eggs

With this experiment we aimed to investigate if female beetle modulate the distances between egg clutches and if they use cognitive capabilities for doing so. Besides the general preparation, we here further standardized the plants in this experiment by additionally removing all leaves except 10 within 25 cm of the shoot. We therefore gained similar distances along the shoot between the leaves. The experimental procedure was the following: (1) in the first part we

allowed each female to lay eggs for four days on the same plant (treatment: *first release*); (2) we measured the distances between the clutches (distance type: *old-old*); (3) for the second part we randomly assigned plants that had received eggs to either of the two treatments: *experienced* (releasing the same female again) or *naïve* (releasing new females that had not yet laid any eggs on any of the plants); (4) after three days all distances between these new clutches (*new-new*) and between the new clutches and the old clutches (*old-new*) were measured. Because the distances between clutches decreased with increasing number of clutches on a plant (adding points in a defined space decrease the mean distances between them; see Paper I) these distances were standardized by dividing each by the number of clutches on the respective plant.

Figure 2: Females were allowed to lay egg clutches (old) where after the distances between these clutches were measured. Then either the same female or a female from the rearing was released again and laid clutches (new) where after the distances between these new clutches (*new-new*) and their relation to the already established clutches (*old-new*) were measured.



4.2 Oviposition in response to host plants and predators

With the following experiments we aimed to understand if predator presences compared to predator absence and host plant quality alters the oviposition behaviour of the leaf beetle.

4.2.1 Clutch size and number of eggs laid on a shoot

First we were interested in the number of eggs that females lay on different plant genotypes and in presence of different predators. This experiment was divided in two parts. The first part was performed in 2009, with a complementary second part in 2015. The first part of this experiment involved all four *Salix* genotypes with either two ovipositing *P. vulgaticissima* females released on individual plants for six days (Control) and one treatment with additionally two *A. nemorum* individuals (2 AN). In the second part of the experiment only the genotypes 78183 and Loden were used and we added the following predators to the plants with ovipositing females: two *O. marginalis*

(2 OM), one *A. nemorum* and one *O. marginalis* (1 AN + 1 OM), or four *O. marginalis* (4 OM). At the end of each experiment the clutch size, the number of consumed eggs, and the position of each clutch were recorded. Because genotypes had different numbers of leaves we divided each shoot into 13 equally sized parts (lowest position was part 1).

4.2.2 Vertical position of eggs on the shoot

In order to understand the behaviour of the leaf beetles we investigated the behaviour of the predators with respect to where they preferentially hunt on the shoot. Because natural oviposition does not result in a homogeneous distribution of eggs on the shoot (Paper IV) we distributed leaf beetle egg clutches along shoots on the *Salix* genotypes Gudrun, Loden, 78183 and 78021. Each plant was again divided into 13 equally sized parts (lowest position was part 1). We then attached leaves that had egg clutches with insect pins on the underside of the leaves of the experimental shoots. These clutches were previously manipulated to three size classes (5, 15, or 45 eggs per clutch). All 13 vertical positions of the experimental plants received one clutch. Three *A. nemorum* individuals or three *O. marginalis* individuals were then allowed to consume eggs for three days, and then we counted the number of empty egg shells and their locations. In addition to the gained information about the preferred vertical hunting area on the shoot, the positions of egg clutches in the previous experiment provided the information on the vertical preferences of the leaf beetles and how it is affected if the predators are present during oviposition.

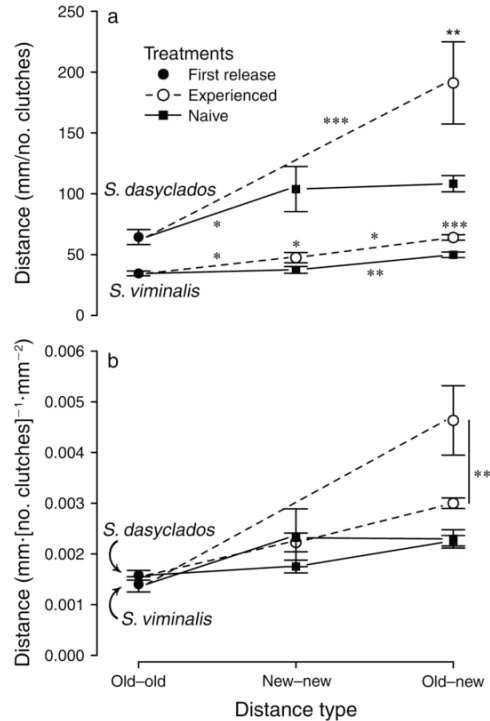
5 Results and Discussion

5.1 Beetle oviposition in presence of conspecific eggs

In this experiment we used the mean distance between clutches to describe the intra-plant clutch distribution because we were interested in the beetle's cognitive capabilities. We found the largest clutch distances for experienced females (Fig. 3) that can rely on perceived cues and memory because they themselves established the already existing clutches on a plant. Because experienced females increased the distances between clutches further than naïve females this behaviour indicates that this species must use some kind of spatial memory (or a cognitive map) to improve its egg distribution. Memory and learning involve costs (Dukas, 2008), but increased fitness due to optimized aggregation should be a strong evolutionary driver and should compensate for these. Because *P. vulgatissima* can memorize spatial information about previous oviposition on a plant it is able to reduce competition between sibling/non-sibling larvae. Because the larvae from nearby clutches will form a group the increase in larval survival would be due to known mechanisms relevant to aggregating organisms (Paper I). In addition, the terms egg pooling/egg clumping/egg clustering that normally refers to eggs that have contact with at least one other conspecific egg could be extended considering this fine-tuned distance modulation. Therefore, arguments for the existence of such modulation should be similar to those related to egg pooling, like host-plant nutrient distribution (Chew & Courtney, 1991) and increased female fecundity (Courtney, 1984).

Intra-plant egg distribution can vary on host plant species due to different suitability (Silva & Furlong, 2012). Here we showed that leaf morphology in itself can also be important, because females increased the distances between clutches on a less suitable host plant due to differences in leaf area and not host suitability (Fig. 3b).

Figure 3: Distances (mean \pm SE) between egg clutches of the leaf beetle on a shoot after (a) correcting for shoots with different numbers of clutches and (b) additional correcting for *Salix* species with different total leaf areas. Largest clutch distances were found among new–new and old–new clutches in part two of the experiment for experienced females that were released on the same respective plants as in part one, and could rely on perceived cues and memory (compare to old–old clutches; old clutches are those laid in part one, new clutches were laid in part two). Naive females released in part two that had not encountered the respective plant before could only rely on cues. Significant differences were determined via a Kolmogorov- Smirnov test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.



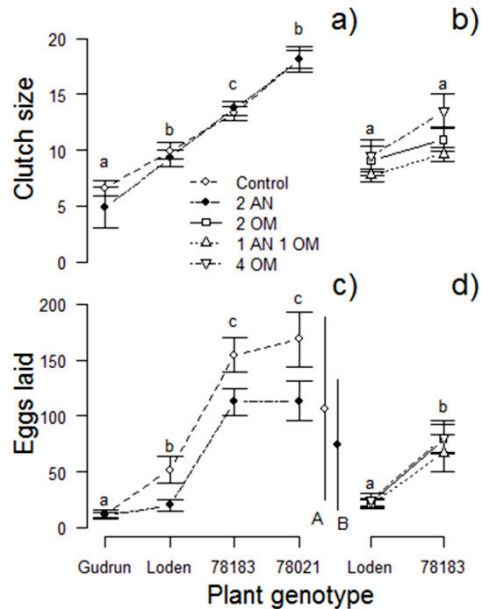
The distance modulations might represent a previously unknown behavioural element of insect oviposition that increases the chances of offspring survival. How exactly these beetles use their memory during oviposition, however, remains unknown. Nevertheless, cognitive capabilities are assumed to alter trophic interactions and population dynamics (Kondoh, 2010), which is probably especially important for gregarious out-breaking species like *P. vulgatissima*. In identifying these new behavioural component of oviposition we so contribute to the mechanistic understanding of the spread of insect outbreaks because population dynamics are shaped by individual decisions.

5.2 Oviposition in response to host plants and predators

5.2.1 Clutch size and number of eggs laid on a shoot

It appears that the mean clutch size of *P. vulgatissima* is driven by the plant genotype because size increases with increasing plant suitability (Fig. 4a, b). This beetle therefore showed similar plasticity found in other herbivorous insects (Pilson & Rausher, 1988), while other may not be able to alter this behavioural trait (Janz & Thompson, 2002). Clutch size may also increase with

Figure 4: Mean (\pm SE) clutch size and eggs laid on individual plants by two *Phratora vulgatissima* females depending on the *Salix* genotype (*S. dasyclados*: Gudrun, Loden; *S. viminalis*: 78183, 78021) and the predator treatment (Control = only leaf beetles, AN = *Anthocoris nemorum*, OM = *Orthotylus marginalis*) for the first (a, c) and second (b, d) experimental part. Lowercase letters indicate differences between genotypes and upper case letters differences between overall means (\pm SD) of treatments ($p < 0.05$; Tukey contrast).



leaf area (Kagata & Ohgushi, 2002) and we showed previously that *P. vulgatissima* increases its distances between clutches on a plant due to higher leaf area of the unsuitable genotype, not the unsuitability itself (Stephan *et al.*, 2015). In contrast, the *S. dasyclados* genotypes (Loden and Gudrun) with twice as large leaves as the *S. viminalis* genotypes (78183 and 78021) received considerably smaller clutches. Although females more frequently initially select larger leaves for oviposition and feeding (Paper IV) there seems to be no relation between clutch size and leaf area of particular leaves and mean clutch size is modulated by plant suitability. Similar to the increase of distances between clutches the adaptive mechanism would be to avoid intraspecific exploitative competition. Before moving to other plant parts the larvae feed gregariously, close to the hatching site, until the 3rd instar. This means that competition is lower on suitable plant genotypes that can support more larvae in an equivalent feeding area (Pilson & Rausher, 1988; Freese & Zwölfer, 1996; Roitberg *et al.*, 1999). Suitable plant genotypes that provide ample food provision therefore sustains larger clutches, suggesting that females match the number of larvae hatching from an egg clutch to the food quality to reduce the risk of larval aggregation problems.

Contrary to the plant genotype *P. vulgatissima* did not follow our expectations regarding the predators. Predator presence did not change the mean clutch size, which was especially surprising for the presence of *A. nemorum* as larger clutch size increase individual egg survival in case where only this predator is foraging on the eggs (Paper II, Paper IV). However,

investigating the actual clutch size distribution showed that either of the predator types/combinations lowered the median and the variation in clutch sizes with smaller sizes becoming more frequent (Paper III). This change in size distribution appeared for both predators meaning the leaf beetle females may not discriminate between the predators.

The other behavioural response becomes apparent by comparing the mean total number of eggs laid on the different genotypes in predator absence and presence (Fig. 4 c, d). We evaluated the oviposition choice with a no-choice assay, not in a field set up (Tschanz *et al.*, 2005) or with alternative host plants. However, oviposition rate is a good indicator for life time fitness in this species and our results confirm previous findings that lower egg numbers are laid on less suitable plant genotypes (Stenberg *et al.*, 2010; Lehrman *et al.*, 2012). We can also conclude that host plant acceptance was due to suitability not larger leaf/feeding area because the cumulative leaf area of all plants was similar.

Most interestingly was the finding that the predator presence also lowers oviposition rate. In the presence of *A. nemorum* the oviposition rate decreased compared to the predator absence treatment and it also seems to decrease in the second part of the experiment for all predator treatments in similar strength. It may be difficult to detect lower oviposition rate on a high predation risk plants in the field (Tschanz *et al.*, 2005) or specific plant genotypes (Stephan *et al.*, 2016) as many other aspects like valuing own performance higher than that of the offspring (Mayhew, 2001), habitat heterogeneity (Andersson *et al.*, 2013), or higher predation risk on otherwise suitable hosts (Egusa *et al.*, 2008) could be more important for egg survival. However, we found first evidence that *P. vulgatissima* could lower its oviposition rate to avoid predation.

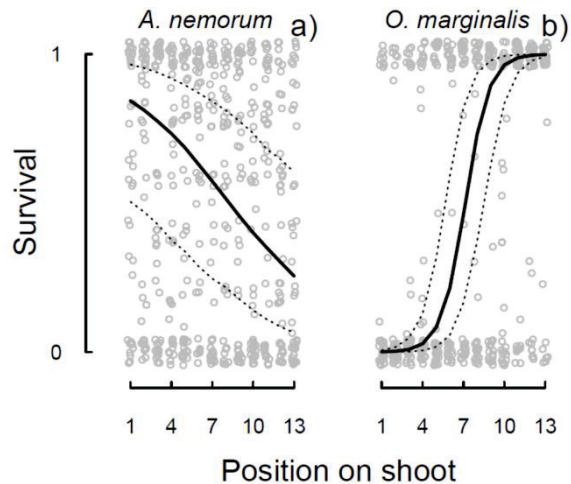
The number of eggs not laid on a plant due to predator presence and plant genotype represent a strong non-consumptive effect (Paper III). As a result, complicated interactions arise because the predators and their consumption of eggs and disturbance of the females are also depending on the plant genotype. For example increased time spent on egg consumption by the predators due to lower plant sap quality is accompanied with less time spent searching for prey and thus disturbing the beetle females less. Consequently, although the plant gains protection through egg consumption by the omnivorous predator, there might be also a “cost” in form of lower benefit from the nonconsumptive effect as the predator will disturb the herbivorous beetles less. This may ultimately increasing damage to plants, depending on the ration between consumptive and nonconsumptive effect on a plant genotype (Paper III). Also, assays investigating indirect defences of plants via ‘bodyguards’ should include foraging kairomones from beetles (Fernandez & Hilker, 2007) or plant volatile induction due to feeding or oviposition (Dicke & Baldwin, 2010). This will

ensure that possible differences in the nonconsumptive effect of the omnivorous predators due to the host plant are included as they may result in differently strong effects on the herbivore reproductive behaviour (Paper III).

5.2.2 Vertical position of eggs on the shoot

We found that both omnivores have contrasting preferred hunting areas at individual plants (Fig. 5). *A. nemorum* mainly consumed eggs in the upper part of the plant, whereas *O. marginalis* preferentially foraged in the lower part. Neither of the four plant genotypes interfered with these contrasting preferences and therefore strengthen our interpretation that these are general attributes. We believe that these different preferences are another important factor contributing to the neutral relationship between the two predator species, which was previously attributed solely to their different foraging strategies (Björkman & Liman, 2005). However, the details of the different preferences remain unexplored and still have to be confirmed in the field. The fact that *A. nemorum* (overwintering as adult) feeds on flower nectar in the shoot canopy (Sigsgaard & Kollmann, 2007) and that *O. marginalis* hatches after willow flowering (overwinters as eggs) and mainly feeds on sap maybe relevant here. Also the very active *A. nemorum* might pass the area occupied by *O. marginalis* as we also have the impression that *O. marginalis* is more territorial because it lays its eggs in the lower part of the shoot and actively defends them. Dominant species are often avoided by less dominant species (Binz *et al.*, 2014) and *A. nemorum* may evade time- and energy-consuming confrontations.

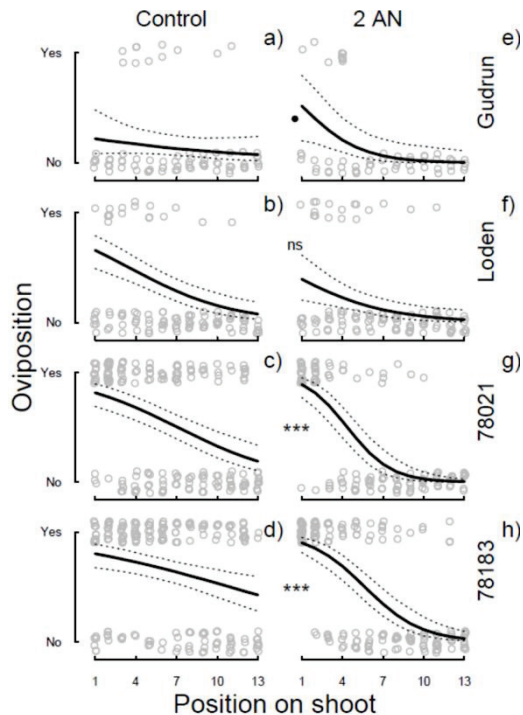
Figure 5: Survival of *Phratora vulgatissima* eggs within a clutch after being exposed to three *Anthocoris nemorum* (a) or three *Orthotylus marginalis* predators (b) in relation to vertical position of the clutch on the shoot (1 = lowest part along the shoot). The survival probability increased down the shoot if *A. nemorum* was released, whereas it increased up the shoot if *O. marginalis* was in the cage. Circles show the proportion survived eggs within clutch (shifted to increase visibility), and the lines indicate the model predictions with bootstrapped confidence limits.



Leaf beetle females preferred to oviposit in the lower part of the shoot, despite the fact that leaves are smaller on the top and bottom of the plant with leaf area also being an important determinant for beetle oviposition (Paper IV). The individual females preferentially feed in the upper shoot part (Paper IV), which may be explained by higher leaf nitrogen concentration in the shoot canopy (Weih & Rönnerberg-Wästjüng, 2007), but move to the lower shoot part for oviposition. This is surprising, and because we may be able to rule out other explanations (Paper IV) we believe this represents predator avoidance behaviour.

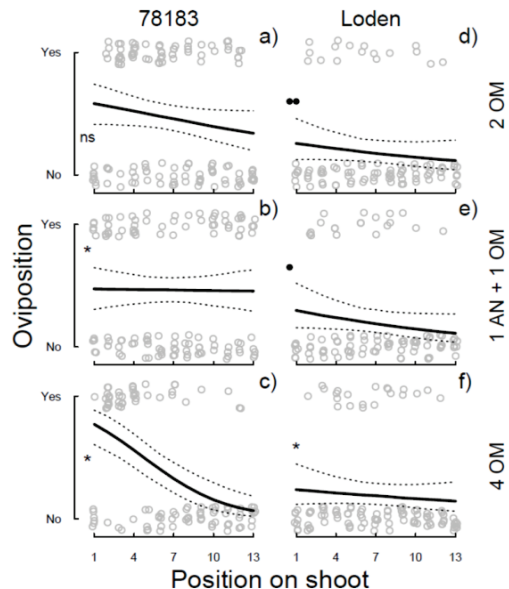
This plastic avoidance was differently strong among predator treatments and plant genotypes. On three of the four plant genotypes we could observe that the presence of *A. nemorum* amplified the behaviour of choosing leaves for oviposition in the lower plant part where this predator is hunting less frequently (Fig. 6). In presence of *O. marginalis*, however, the leaf beetles did not respond strongly (Fig. 7a, d). This may be due to the previously mentioned shorter temporary overlap early in the spring in combination with the believe that time to first reproduction and the survival of herbivore eggs laid early in the season are very important (Parry *et al.*, 1998). Additionally, the lower mobility of *O. marginalis* may lead to less frequent encounters by the beetles compared to *A. nemorum* with its ‘run and eat’ behaviour. The shoot canopy

Figure 6: Oviposition of two *Phratora vulgatissima* females in relation to vertical position on the shoot (1 = lowest part along the shoot) with respect to different predator treatments (Control = only leaf beetles females, 2 AN = leaf beetles and two *Anthocoris nemorum* individuals) and plant genotype (*Salix dasyclados*: Gudrun, Loden; *Salix viminalis*: 78021, 78183). Circles show the incidence of oviposition (shifted to increase visibility), and the lines show the model predictions with bootstrapped confidence limits (comparisons to slope of respective Control: *** = $p < 0.001$; ● = $p < 0.09$; ns = $p \geq 0.05$).



may therefore appear more dangerous and the fact that alarm substances produce by Anthocoridae (Evans, 1976), that may be used by the leaf beetle as a foraging kairomone, could contribute to this interpretation. In confronting the ovipositing leaf beetles with both predators simultaneously we found a tendency for a weakened preference on Loden and no position preference on 78183 (Fig. 7b, e). This last result validates that the females are able to perceive both predators, try to avoid their respective habitat domains, and anticipate future predation on their eggs/larvae because the predators are not harmful to the adult females themselves. For the last treatment we doubled the number of *O. marginalis* and we expected that the leaf beetles preference for the lower shoot part would be even more weakened. This was true for the genotype Loden, but not 78183 where we observed the exact opposite. This interaction between predator density and plant genotype is puzzling at the moment and will be investigated further.

Figure 7: Oviposition of two *Phratora vulgatissima* females in relation to vertical position on the shoot (1 = lowest part along the shoot) with respect to plant genotype (*Salix dasyclados*: Loden; *Salix viminalis*: 78183) and different predator treatments (Control = only leaf beetles (see Fig. 4b and 4d, respectively), AN = *Anthocoris nemorum*, OM = *Orthotylus marginalis*). Circles show the incidence of oviposition (shifted to increase visibility), and the lines show the model prediction with bootstrapped confidence limits (comparisons to slope of respective Control: * = $p < 0.05$; ●● = $p < 0.06$; ● = $p < 0.09$; ns = $p \geq 0.05$).



6 Conclusion

We showed that the leaf beetle *P. vulgatissima* possesses sophisticated strategies to respond to a changing environment during egg laying. The changes in the environment(s) are set by the presence of conspecifics on host plant and its quality, the predators that are present during oviposition, and the direct and indirect interactions among these tree factors within this tritrophic system. It is believed that one element that may contribute to the occurrence of insect outbreaks is gregariousness because this form of living is shared among most outbreaking species. Furthermore, due to the number and complexity of mechanisms accompanied, it is believed that this form of living increases the variability of survival chances making insect outbreaks and their causes hard to explain or even predict. We illustrated which factors might modulate this variability and explored some behavioural mechanisms of the outbreaking herbivore and two omnivorous predators. We hope we contributed to the understanding on group living behaviour and (may) inspired new interesting ecological questions.

References

- Agrawal, A. a (2007). Macroevolution of plant defense strategies. *Trends in ecology & evolution*, 22(2), pp 103–9.
- Andersson, P., Löfstedt, C. & Hambäck, P. a. (2013). Insect density-plant density relationships: A modified view of insect responses to resource concentrations. *Oecologia*, 173(4), pp 1333–1344.
- Bailey, J. K., Wooley, S. C., Lindroth, R. L. & Whitham, T. G. (2006). Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecology letters*, 9(1), pp 78–85.
- Beckerman, A. P., Uriarte, M. & Schmitz, O. J. (1997). Experimental evidence for a behavior-mediated trophic cascade in a terrestrial food chain. *Proceedings of the National Academy of Sciences of the United States of America*, 94(20), pp 10735–10738 The National Academy of Sciences of the USA.
- Bergstrom, A., Janz, N. & Nylin, S. (2006). Putting more eggs in the best basket: clutch-size regulation in the comma butterfly. *Ecological Entomology*, 31(3), pp 255–260.
- Binz, H., Foitzik, S., Staab, F. & Menzel, F. (2014). The chemistry of competition: exploitation of heterospecific cues depends on the dominance rank in the community. *Animal Behaviour*, 94, pp 45–53 Elsevier Ltd.
- Bitume, E., Bonte, D., Ronce, O. & Bach, F. (2013). Density and genetic relatedness increase dispersal distance in a subsocial organism. *Ecology Letters*, 16(4), pp 430–437.
- Björkman, C., Bommarco, R., Eklund, K. & Höglund, S. (2004). Harvesting disrupts biological control of herbivores in a short-rotation coppice system. *Ecological Applications*, 14(6), pp 1624–1633 Eco Soc America.
- Björkman, C., Dalin, P. & Eklund, K. (2003). Generalist natural enemies of a willow leaf beetle (*Phratora vulgatissima*): abundance and feeding habits. *Journal of Insect Behavior*, 16(6), pp 747–764.
- Björkman, C. & Eklund, K. (2006). Factors affecting willow leaf beetles (*Phratora vulgatissima*) when selecting overwintering sites. *Agricultural and Forest Entomology*, 8(2), pp 97–101.
- Björkman, C., Höglund, S., Eklund, K. & Larsson, S. (2000). Effects of leaf beetle damage on stem wood production in coppicing willow. *Agricultural and*

- Forest Entomology*, 2(2), pp 131–139 Wiley Online Library.
- Björkman, C. & Liman, A.-S. (2005). Foraging behaviour influences the outcome of predator-predator interactions. *Ecological Entomology*, 30(2), pp 164–169.
- Chew, F. S. & Courtney, S. P. (1991). Plant apparency and evolutionary escape from insect herbivory. *The American Naturalist*, 138(3), pp 729–750.
- Clark, B. & Faeth, S. (1997). The consequences of larval aggregation in the butterfly *Chlosyne lacinia*. *Ecological Entomology*, 22(4), pp 408–415.
- Collett, M., Chittka, L. & Collett, T. (2013). Spatial memory in insect navigation. *Current Biology*, 23(17), pp 789–800 Elsevier.
- Courtney, S. (1984). The evolution of egg clustering by butterflies and other insects. *The American Naturalist*, 123(2), pp 276–281.
- Dalin, P. (2006). Habitat difference in abundance of willow leaf beetle *Phratora vulgatissima* (Coleoptera: Chrysomelidae): plant quality or natural enemies? *Bulletin of entomological research*, pp 629–635.
- Dalin, P. (2011). Diapause induction and termination in a commonly univoltine leaf beetle (*Phratora vulgatissima*). *Insect Science*, 18(4), pp 443–450.
- Degen, T., Dillmann, C., Marion-Poll, F. & Turlings, T. C. J. (2004). High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. *Plant physiology*, 135(4), pp 1928–38.
- Denno, R. & Benrey, B. (1997). Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. *Ecological Entomology*, 22(2), pp 133–141 Wiley Online Library.
- Dicke, M. & Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the "cry for help". *Trends in Plant Science*, 15(3), pp 167–75 Elsevier Ltd.
- Dukas, R. (2008). Evolutionary biology of insect learning. *Annual Review of Entomology*, 53, pp 145–60.
- Egusa, S., Nishida, T., Sawada, H. & Fujisaki, K. (2008). Is selection of host plants by *Plagioderaversicolora* based on plant-related performance? *Entomologia Experimentalis et Applicata*, 128(2), pp 258–264.
- Eubanks, M. D. & Denno, R. F. (1999). The ecological consequences of variation in plants and prey for an omnivorous insect. *Ecology*, 80(4), pp 1253–1266.
- Evans, H. F. (1976). Mutual interference between predatory anthocorids. *Ecological Entomology*, 1(4), pp 283–286.
- Fernandez, P. & Hilker, M. (2007). Host plant location by Chrysomelidae. *Basic and Applied Ecology*, 8(2), pp 97–116.
- Freese, G. & Zwölfer, H. (1996). The problem of optimal clutch size in a tritrophic system: the oviposition strategy of the thistle gallfly *Urophora cardui* (Diptera, Tephritidae). *Oecologia*, 108(2), pp 293–302.
- Godfray, H. C. J. (1986). Clutch size in a leaf-mining fly (*Pegomya nigrotarsis*: Anthomyiidae). *Ecological Entomology*, 11, pp 75–81.
- Godfray, H. C. J., Partridge, L. & Harvey, P. H. (1991). Clutch Size. *Annual Review of Ecology and Systematics*, 22(1), pp 409–429.
- Le Goff, G., Mailleux, A.-C., Detrain, C., Deneubourg, J.-L., Clotuche, G. &

- Hance, T. (2009). Spatial distribution and inbreeding in *Tetranychus urticae*. *Comptes Rendus Biologies*, 332(10), pp 927–933 Elsevier Masson SAS.
- Harfenist, A. & Ydenberg, R. (1995). Parental provisioning and predation risk in rhinoceros auklets (*Cerorhinca monocerata*): effects on nestling growth and fledging. *Behavioral Ecology*, 6(1), pp 82–86.
- Heisswolf, A., Poethke, H. & Obermaier, E. (2006). Multitrophic influences on egg distribution in a specialized leaf beetle at multiple spatial scales. *Basic and Applied Ecology*, 7(6), pp 565–576.
- Held, D. W., Eaton, T. & Potter, D. A. (2001). Potential for habituation to a neem-based feeding deterrent in Japanese beetles, *Popillia japonica*. *Entomologia Experimentalis et Applicata*, 101(1), pp 25–32.
- Hemptinne, J. L., Dixon, A. F. G. & Coffin, J. (1992). Attack strategy of ladybird beetles (Coccinellidae): factors shaping their numerical response. *Oecologia*, 90(2), pp 238–245.
- Higashiura, Y. (1989). Survival of eggs in the gypsy moth *Lymantria dispar*. II. Oviposition site selection in changing environments. *The Journal of Animal Ecology*, 58(2), pp 413–426.
- Hunter, a F. (2000). Gregariousness and repellent defences in the survival of phytophagous insects. *Oikos*, 91(2), pp 213–224.
- Janz, N. & Thompson, J. (2002). Plant polyploidy and host expansion in an insect herbivore. *Oecologia*, 130(4), pp 570–575.
- Joos, B., Casey, T., Fitzgerald, T. & Buttemer, W. (1988). Roles of the tent in behavioral thermoregulation of eastern tent caterpillars. *Ecology*, 69(6), pp 2004–2011.
- Kagata, H. & Ohgushi, T. (2002). Clutch size adjustment of a leaf-mining moth (Lyoniidae: Lepidoptera) in response to resource availability. *Ecology and Population Biology*, 95(2), pp 213–217.
- Kaplan, I. & Thaler, J. S. (2010). Plant resistance attenuates the consumptive and non-consumptive impacts of predators on prey. *Oikos*, 119(7), pp 1105–1113.
- Karban, R. (2011). The ecology and evolution of induced resistance against herbivores. *Functional Ecology*, 25(2), pp 339–347.
- Karp, A. & Shield, I. (2008). Bioenergy from plants and the sustainable yield challenge. *New Phytologist*.
- Kendall, D., Wiltshire, C. & Butcher, M. (1996). Phenology and population dynamics of willow beetles (Coleoptera; Chrysomelidae) in short-rotation coppiced willows at Long Ashton. *ETSU (DTI) Biofuels Study B/M4/00487/14/REP. IACR Long Ashton Research Station, Long Ashton, Bristol.*
- Keoleian, G. A. & Volk, T. A. (2005). Renewable Energy from Willow Biomass Crops: Life Cycle Energy, Environmental and Economic Performance. *Critical Reviews in Plant Sciences* Taylor & Francis.
- Kersch-Becker, M. F. & Thaler, J. S. (2015). Plant resistance reduces the strength of consumptive and non-consumptive effects of predators on aphids. *Journal of Animal Ecology*, pp 1222–1232.

- Kessler, A. & Baldwin, I. (2002). *Manduca quinquemaculata*'s optimization of intra-plant oviposition to predation, food quality, and thermal constraints. *Ecology*, 83(8), p 2346.
- Kondoh, M. (2010). Linking learning adaptation to trophic interactions: a brain size-based approach. *Functional Ecology*, 24(1), pp 35–43.
- Lack, D. (1947). The significance of clutch size. *Ibis*, 89(2), pp 302–352.
- Lee, D. H., Nyrop, J. P. & Sanderson, J. P. (2014). Non-consumptive effects of the predatory beetle *Delphastus catalinae* (Coleoptera: Coccinellidae) on habitat use patterns of adult whitefly *Bemisia argentifolii* (Hemiptera: Aleyrodidae). *Applied Entomology and Zoology*, 49(4), pp 599–606.
- Lehrman, A., Torp, M., Stenberg, J. A., Julkunen-Tiitto, R. & Björkman, C. (2012). Estimating direct resistance in willows against a major insect pest, *Phratora vulgatissima*, by comparing life history traits. *Entomologia Experimentalis et Applicata*, 144(1), pp 93–100.
- Loeb, M., Diener, L. & Pfennig, D. (2000). Egg-dumping lace bugs preferentially oviposit with kin. *Animal Behaviour*, 59(2), pp 379–383.
- Lundgren, J. G., Hesler, L. S., Tilmon, K., Dashiell, K. & Scott, R. (2009). Direct effects of soybean varietal selection and *Aphis glycines*-resistant soybeans on natural enemies. *Arthropod-Plant Interactions*, 3(1), pp 9–16.
- Matassa, C. M. & Trussell, G. C. (2011). Landscape of fear influences the relative importance of consumptive and nonconsumptive predator effects. *Ecology*, 92(12), pp 2258–2266 Ecological Society of America.
- Matsumoto, K. (1990). Population dynamics of *Luehdorfia japonica* Leech (Lepidoptera : Papilionidae). II. Patterns of mortality in immatures in relation to egg cluster size. *Researches on Population Ecology*, 32, pp 173–188.
- Mayhew, P. J. (2001). Herbivore host choice and optimal bad motherhood. *Trends in ecology & evolution*, 16(4), pp 165–167.
- Meiners, T., Hacker, N. K., Anderson, P. & Hilker, M. (2005). Response of the elm leaf beetle to host plants induced by oviposition and feeding: the infestation rate matters. *Entomologia Experimentalis et Applicata*, 115(1), pp 171–177.
- Meiners, T. & Obermaier, E. (2004). Hide and seek on two spatial scales—vegetation structure effects herbivore oviposition and egg parasitism. *Basic and Applied Ecology*, 5(1), pp 87–94.
- Miller, J. R. B., Ament, J. M. & Schmitz, O. J. (2014). Fear on the move: predator hunting mode predicts variation in prey mortality and plasticity in prey spatial response. *Journal of Animal Ecology*, 83(1), pp 214–222.
- Mitchell, R. (1975). The evolution of oviposition tactics in the bean weevil, *Callosobruchus maculatus* (F.). *Ecology*, 56(3), pp 696–702.
- Mulatu, B., Applebaum, S. W. & Coll, M. (2006). Effect of tomato leaf traits on the potato tuber moth and its predominant larval parasitoid: A mechanism for enemy-free space. *Biological Control*, 37(2), pp 231–236.
- Navasero, R. & Ramaswamy, S. (1993). Influence of plant age, water stress, larval damage, and presence of conspecific eggs on oviposition by *Heliothis virescens* (F.) on cotton. *Journal of Applied Entomology*, 115(1-5), pp 97–106.

- Nelson, E. H. (2007). Predator avoidance behavior in the pea aphid: costs, frequency, and population consequences. *Oecologia*, 151(1), pp 22–32.
- Obermaier, E. & Zwölfer, H. (1999). Plant quality or quantity? Host exploitation strategies in three Chrysomelidae species associated with Asteraceae host plants. *Entomologia Experimentalis et Applicata*, 92(2), pp 165–177.
- Parry, D., Spence, J. & Volney, W. (1998). Budbreak phenology and natural enemies mediate survival of first-instar forest tent caterpillar (Lepidoptera: Lasiocampidae). *Environmental Entomology*, 27(6), pp 1368–1374.
- Peacock, L. & Herrick, S. (2000). Responses of the willow beetle *Phratora vulgatissima* to genetically and spatially diverse *Salix* spp. plantations. *Journal of Applied Ecology*, 37(5), pp 821–831.
- Peacock, L., Lewis, M. & Herrick, S. (2001). Factors influencing the aggregative response of the blue willow beetle, *Phratora vulgatissima*. *Entomologia Experimentalis et Applicata*, 98(2), pp 195–201.
- Pilson, D. & Rausher, M. D. (1988). Clutch size adjustment by a swallowtail butterfly. *Nature*, 333(6171), pp 361–363 Nature Publishing Group.
- Preisser, E. L., Bolnick, D. I. & Benard, M. F. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, 86(2), pp 501–509 Eco Soc America.
- Raitanen, J., Forsman, J. T., Kivela, S. M., Maenpaa, M. I. & Valimaki, P. (2013). Attraction to conspecific eggs may guide oviposition site selection in a solitary insect. *Behavioral Ecology*, 25(1), pp 110–116.
- Rausher, M. D. (1983). Conditioning and genetic variation as causes of individual variation in the oviposition behaviour of the tortoise beetle, *Deloyala guttata*. *Animal Behaviour*, 31(3), pp 743–747.
- Refsnider, J. M. & Janzen, F. J. (2010). Putting eggs in one basket: Ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annual Review of Ecology, Evolution, and Systematics*, 41(1), pp 39–57.
- Roitberg, B. D., Robertson, I. C. & Tyerman, J. G. A. (1999). Vive la variance : a functional oviposition theory for insect herbivores. *Annual Review of Entomology*, (1978), pp 187–194.
- Schaller, A. (2008). *Induced plant resistance to herbivory*. Springer Verlag.
- Schmitz, O., Grabowski, J. & Peckarsky, B. (2008). From individuals to ecosystem function: toward an integration of evolutionary and ecosystem ecology. *Ecology*, 89(9), pp 2436–2445.
- Schmitz, O. J., Krivan, V. & Ovadia, O. (2004). Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecology Letters*, 7(2), pp 153–163.
- Siemens, D. & Johnson, C. (1992). Density-dependent egg parasitism as a determinant of clutch size in bruchid beetles (Coleoptera: Bruchidae). *Environmental Entomology*, 21(3), pp 610–619.
- Sigsgaard, L. & Kollmann, J. (2007). Beneficial effects of hedgerow plants for insect predators in adjacent orchards-the value of pollen and nectar to *Anthocoris nemorum* (L.). *IOBC WPRS BULLETIN*, 30(4), pp 4–6.
- Silva, R. & Furlong, M. J. (2012). Diamondback moth oviposition: effects of host plant and herbivory. *Entomologia Experimentalis et Applicata*, 143(3), pp

218–230.

- Srinivasan, M. V. (2010). Honey bees as a model for vision, perception, and cognition. *Annual Review of Entomology*, 55, pp 267–84.
- Stenberg, J. A., Lehrman, A. & Björkman, C. (2010). Uncoupling direct and indirect plant defences: Novel opportunities for improving crop security in willow plantations. *Agriculture, Ecosystems and Environment*, 139(4), pp 528–533.
- Stenberg, J. a., Lehrman, A. & Björkman, C. (2011a). Host-plant genotype mediates supply and demand of animal food in an omnivorous insect. *Ecological Entomology*, 36(4), pp 442–449.
- Stenberg, J. A., Lehrman, A. & Björkman, C. (2011b). Plant defence: Feeding your bodyguards can be counter-productive. *Basic and Applied Ecology*, 12(7), pp 629–633 Elsevier GmbH.
- Stephan, J. G., Albertsson, J., Wang, L. & Porcel, M. (2016). Weeds within willow short-rotation coppices alter the arthropod community and improve biological control of the blue willow beetle. *BioControl*, 61(1), pp 103–114.
- Stephan, J. G., Stenberg, J. A. & Björkman, C. (2015). How far away is the next basket of eggs? Spatial memory and perceived cues shape aggregation patterns in a leaf beetle. *Ecology*, 96(4), pp 908–914.
- Subinprasert, S. & Svensson, B. W. (1988). Effects of predation on clutch size and egg dispersion in the codling moth *Laspeyresia pomonella*. *Ecological Entomology*, 13, pp 87–94.
- Tack, A. J. M., Ovaskainen, O., Pulkkinen, P. & Roslin, T. (2010). Spatial location dominates over host plant genotype in structuring an herbivore community. *Ecology*, 91(9), pp 2660–2672.
- Tanmaru, T., Kaitaniemi, P. & Ruohomäki, K. (1996). Realized fecundity in *Epirrita autumnata* (Lepidoptera: Geometridae): Relation to body size and consequences to population dynamics. *Oikos*, 77(3), pp 407–416.
- Thaler, J. S., Contreras, H. & Davidowitz, G. (2014). Effects of predation risk and plant resistance on *Manduca sexta* caterpillar feeding behaviour and physiology. *Ecological Entomology*, 39(2), pp 210–216.
- Travers, S. E. & Sih, A. (1991). The influence of starvation and predators on the mating behavior of a semiaquatic insect. *Ecology*, 72(6), pp 2123–2136.
- Trussell, G. C., Ewanchuk, P. J. & Bertness, M. D. (2003). Trait-mediated effects in rocky intertidal food chains: Predator risk cues alter prey feeding rates. *Ecology*, 84(3), pp 629–640 ECOLOGICAL SOC AMER.
- Tschanz, B., Schmid, E. & Bacher, S. (2005). Host plant exposure determines larval vulnerability - do prey females know? *Functional Ecology*, 19(3), pp 391–395.
- Underwood, N. & Rausher, M. (2000). The effects of host-plant genotype on herbivore population dynamics. *Ecology*, 81(6), pp 1565–1576.
- Unsicker, S. B., Alexandra, F., Juliane, S., G??nter, K., Jeanine, L., Carsten, R., Claudia, S. & Weisser, W. W. (2010). Plant species richness in montane grasslands affects the fitness of a generalist grasshopper species. *Ecology*, 91(4), pp 1083–1091.

- Uzendoski, K., Maksymovitch, E. & Verrell, P. (1993). Do the Risks of Predation and Intermale Competition Affect Courtship Behavior in the Salamander *Desmognathus ochrophaeus*. *Behavioral Ecology and Sociobiology*, 32(6), pp 421–427.
- Vasconcellos-Neto, J. & Monteiro, R. F. (1993). Inspection and evaluation of host plant by the butterfly *Mechanitis lysimnia* (Nymph., Ithomiinae) before laying eggs: a mechanism to reduce intraspecific competition. *Oecologia*, 95(3), pp 431–438.
- Vonesh, J. & Blaustein, L. (2010). Predator-induced shifts in mosquito oviposition site selection: a meta-analysis and implications for vector control. *Israel Journal of Ecology & Evolution*, 56(3-4), pp 263–279.
- Weih, M. & Rönnerberg-Wästjüng, A.-C. (2007). Shoot biomass growth is related to the vertical leaf nitrogen gradient in *Salix* canopies. *Tree physiology*, 27(11), pp 1551–1559.
- Whitham, T. (1978). Habitat selection by *Pemphigus* aphids in response to response limitation and competition. *Ecology*, 59(6), pp 1164–1176.
- Wimp, G. M., Murphy, S. M., Finke, D. L., Huberty, A. F. & Denno, R. F. (2010). Increased primary production shifts the structure and composition of a terrestrial arthropod community. *Ecology*, 91(11), pp 3303–3311 *Eco Soc America*.
- Wright, G. & Schiestl, F. (2009). The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Functional Ecology*, 23(5), pp 841–851.
- Young, A. & Moffett, M. (1979). Studies on the population biology of the tropical butterfly *Mechanitis isthmia* in Costa Rica. *American Midland Naturalist*, 101(2), pp 309–319.
- Zink, A. G. (2003). Intraspecific brood parasitism as a conditional reproductive tactic in the treehopper *Publilia concava*. pp 406–415.

Acknowledgements

I thank Christer Björkman and Johan Stenberg, the best supervisors I could imagine, for their very valuable help and guidance during my thesis and most importantly let me develop and follow my interests in the field of ecology. I also thank Karl Gotthard for agreeing to be part of the supervisor team, although we did not have the opportunity to apply his knowledge and experience (until now). Many thanks go to Mikael Andersson Franko and Matt Low for helping to advance my statistical knowledge and skills and I can only warmly agree on Karin Eklund getting the prize for best technical-administrative staff. Many thanks also to the people that read and commented the thesis and the manuscripts.

Because the risk of not mentioning anyone by name is too great I generally thank everybody in our great and cosy house of science for the last years. I always felt very at home and enjoyed meeting colleagues and friends at coffee breaks and lunch. Even more I am thankful I was surrounded by a vibrating group of people always coming up with great plans to spend time after work.

No thanks would be complete without highlighting my parents, and near and far friends that always encouraged me finding my way. Especially my wife Katharina that went all the way to Sweden with me and blessed us with a wonderful daughter I am most grateful to.

I thank all of you, and if I ever get a (funky) time machine I would do it all again exactly.

How far away is the next basket of eggs? Spatial memory and perceived cues shape aggregation patterns in a leaf beetle

JÖRG G. STEPHAN,^{1,3} JOHAN A. STENBERG,² AND CHRISTER BJÖRKMAN¹

¹Department of Ecology, Unit of Forest Entomology, Swedish University of Agricultural Sciences, P.O. Box 7044, SE-75007 Uppsala, Sweden

²Department of Plant Protection Biology, Unit of Integrated Plant Protection, Swedish University of Agricultural Sciences, P.O. Box 102, SE-23053 Alnarp, Sweden

Abstract. Gregarious organisms need to handle the trade-off between increasing food competition and the positive effects of group living, and this is particularly important for ovipositing females. We hypothesized that insect females consider how many conspecifics previously visited a host plant. In a no-choice assay, we show that the gregarious blue willow leaf beetle (*Phratora vulgatissima*) laid the most eggs and the largest clutches on plants where a sequence of few individual females was released, compared to plants where one or many different females were repeatedly released. Therefore, this species is more sensitive to the indirectly perceived number of conspecifics than the directly perceived number of eggs on a plant. We further hypothesized that females adjust their own intra-plant egg clutch distribution to that of conspecifics and discovered a new behavioral component, i.e., the modulation of distances between clutches. Females adjusted these distances in ways indicating the use of spatial memory, because the largest distance increases were observed on plants with their own clutches, compared to plants with clutches from conspecifics. However, adjustment of aggregation level and distance between clutches occurred only on a suitable, and not on an unsuitable, *Salix* genotype. We conclude that both behaviors should reduce competition between sibling and non-sibling larvae.

Key words: blue willow leaf beetle, *Phratora vulgatissima*; clutch size; cognitive map; Coleoptera; density; distance; egg pooling; kin recognition; learning; relatedness; *Salix*.

INTRODUCTION

The distribution of individuals should reflect habitat quality, where quality itself partly depends on the inhabitant density (Fretwell 1969). Ideally, individuals should achieve equal fitness because the costs of intraspecific competition are divided equally among them. Yet, empirical (Doligez et al. 2002) and theoretical (Folmer et al. 2012) studies show that suitable patches are left unoccupied due to conspecific attraction leading to aggregations. How these aggregations are formed and which behavioral components are involved is of great interest in many systems.

Female herbivorous insects should choose the most suitable spot for their progeny (Jaenike 1978, Gripenberg et al. 2010). Larval survival normally decreases with increasing egg number due to exploitative competition (Mitchell 1975) and increased predation risks while searching for alternative feeding sites (Matsumoto 1990). Larvae are believed to aggregate to increase predator protection (Denno and Benrey 1997, Hunter 2000), overcome plant defenses (Clark and Faeth 1997), and improve thermoregulation (Joos et al. 1988). Two

behavioral components lead to larval aggregation. The first is the choice of where to place the eggs within the landscape (Heisswolf et al. 2006) or plant (Whitham 1978, Silva and Furlong 2012), and can be modulated by predators/parasitoids (Higashiura 1989), the availability and quality of host plants (Obermaier and Zwölfer 1999), and vegetation structure (Meiners and Obermaier 2004). Information about conspecific egg presence might also influence the oviposition choice, but this phenomenon has not been sufficiently investigated. Some species prefer egg-free hosts (Vasconcellos-Neto and Monteiro 1993), some prefer the presence of conspecific eggs (Navasero and Ramaswamy 1993, Raitanen et al. 2013), and some seem to balance larval feeding competition and parasitism threats (Meiners et al. 2005). The second component is the clutch size decision. How many eggs to oviposit in a clutch can depend on resource size (Godfray 1986), resource quality (Bergstrom et al. 2006), and predation/parasitism (Subinprasert and Svensson 1988, Siemens and Johnson 1992).

Siblings/non-siblings from different clutches often share resources and, therefore, face similar predation and competition as progeny from a single clutch. As with Lack's clutch-size hypothesis (Lack 1947, Godfray et al. 1991), which describes the optimal clutch size that produces the highest progeny survival, one could imagine an optimal distance between clutches as an

Manuscript received 15 June 2014; revised 19 November 2014; accepted 26 November 2014. Corresponding Editor: J. T. Cronin.

³ E-mail: jorg.stephan@slu.se

additional behavioral component for determining a suitable aggregation level. Recent studies on kin recognition by spider mites have revealed that the dispersal distance in a population can be driven by relatedness (Bitume et al. 2013), and that the spatial distribution of adults on a plant increases with inbreeding (Le Goff et al. 2009). Predatory mites have also been shown to put greater distances between eggs of more distantly related conspecifics (Faraji et al. 2000). Some insects can recognize eggs from conspecifics (Loeb et al. 2000, Zink 2003), but how this affects distances between clutches is unknown.

Both the direct perception of eggs and indirect cues could inform females about the occupancy of a plant by conspecifics. Sex pheromones, aggregation pheromones, feces, and larval defense secretions (Fernandez and Hilker 2007), as well as induced plant volatiles (Dicke and Baldwin 2010), play important roles in determining host suitability and likely affect aggregation behavior. At the same time, insects are able to learn and memorize, but most studies deal with ants, bees, parasitoid wasps, fruit flies, and grasshoppers (Dukas 2008, Wright and Schiestl 2009). Studies on beetles have focused on conditioning (Held et al. 2001) and how larval experience influences adult behavior (Rausher 1983), but while much is known about the spatial memory of bees, wasps, and ants (Srinivasan 2010, Collett et al. 2013) nothing is known about the spatial memory of beetles.

Here, we studied the gregarious blue willow leaf beetle *Phratora vulgatissima* (Coleoptera: Chrysomelidae), which frequently reaches outbreak densities in natural willow stands and plantations (Björkman et al. 2000, Dalin et al. 2009). When beetles begin to lay eggs, more and more beetles aggregate and the area of infestation increases (C. Björkman, unpublished data). Ovipositing females, especially at the edge of the infestation, will switch to other plants at some level of conspecific occupancy to avoid drastic decreases in host-plant quality. This study aimed to determine when females will no longer accept already-occupied hosts and whether indirect perception of conspecifics affects this decision. Hence, we go beyond a simple egg/adult density-dependent response approach and examine whether the number of conspecifics on a host plant provides females with additional indirectly perceived information. The aim was not to identify the cues of importance, just to determine if any cues exist. We hypothesized in a first experiment that females would show lower host acceptance (fewer eggs) if they were the only ones on the plant and their choice was not confirmed by the presence of conspecifics, or if they faced an overwhelming number of conspecifics that were indicative of high exploitative competition.

In a second experiment, we investigated whether females modulate the distance between clutches and if they use spatial memory during this intra-plant clutch distribution. We expected to see increases in distances between clutches, because this would reduce exploitative

competition among larvae. We hypothesized that females will establish larger distances among their clutches compared to the already-established old clutches on a plant and will lay their new clutches farther away from these old clutches. If the female is the one that established the old clutches, distances should increase even more, since this female can rely on perceived cues and memory. Because hybrid *Salix* genotypes are morphologically and chemically different (Lehrman et al. 2012), not all are equally suitable for this leaf beetle (Stenberg et al. 2010). Therefore, we tested if there is an interaction between host occupancy/clutch distance modulation and host genotype. We expected that host occupancy would become less important if the genotype is a poor host, and that clutch distances would become greater simply due to larger leaves and/or due to lower plant genotype suitability because each larva then needs more leaf area.

MATERIALS AND METHODS

Study system and general set up

Phratora vulgatissima [L.] adults and larvae skeletonize willow leaves (*Salix* spp.), and these beetles are the most common specialist herbivore of willow in Europe (Peacock and Herrick 2000). Adults overwinter in reeds or under the bark of trees (Björkman and Eklund 2006), emerge in April, feed for about two weeks, mate, and subsequently lay hundreds of eggs on the undersides of leaves in clutches of 1–50 eggs. Hatching larvae feed gregariously during the first and second instar and then solitarily on different leaves during the third instar (Kendall et al. 1996). This is followed by pupation in the soil. Adults emerge in August, feed quickly, and find hibernation sites. Adults probably excrete pheromones that attract other individuals (Peacock et al. 2001).

We grew *Salix* shoots from 20-cm winter cuttings (suitable host [Sv], *S. viminalis* genotype 78183; unsuitable host [Sd], *S. dasycladus* genotype Gudrun) that were placed in cylindrical transparent plastic cages (70 cm height, 30 cm diameter) with a net on top. Beetles were collected in the Uppsala, Sweden area and reared in cages on Sv or *S. cinerea* (a suitable native host). Individuals used in this study consisted of a mixture of field-collected and next-generation beetles from both rearing cages that were randomly distributed over the experiments/treatments. All experiments were performed in a greenhouse (23°C, relative humidity of 80%, light regime 18 h light : 6 h dark).

Experiment 1: Influence of host occupancy level on host acceptance

Here, we investigated host acceptance modulation, and attempted to manipulate not the conspecific density but the number of indirectly perceived other females. At least three days before the start (to exclude wound responses), plants (~60 cm height) were modified by removing side shoots, dried leaves, and leaves not fully expanded at the top. At the start, one female was

released onto each plant. Every morning for nine days, the female was removed for ~5 min (time required for catch/release) from the plant it currently occupied and released again based on one of the following three routines forming the treatments: on the same plant again (same host; Sv $N = 10$ plants; Sd $N = 7$ plants), on the next plant among the plants in that treatment (new hosts; Sv $N = 10$ plants; Sd $N = 5$ plants); or onto either the same or the next plant (mixed hosts; Sv $N = 15$ plants; Sd $N = 3$ plants). The five changes to the next plant for mixed hosts were distributed over time and followed the pattern 10110101 (1 signifies change to next plant; 0 signifies same plant again). With these treatments, we achieved plants where females had laid eggs while the number of conspecifics on these plants increased differently (Appendix A). The mixed hosts treatment is most likely closest to resembling the gregarious laying of some eggs followed by moving to another plant. Using the very unsuitable Sd genotype led to the escape of many beetles. Thus, we only used plants that were visited by the desired total number of beetles within each treatment, and this explains the differences in replication numbers. Herbivory can affect host acceptance (Meiners et al. 2005), but this was similar between treatments (only one beetle at a time).

Experiment 2: Influence of cues and memory on clutch distance modulation

Here, we were interested in the clutch distances and the cognitive capability of beetles. Several days before the experiment, we standardized the plants by removing upper and lower leaves and unwanted leaves within the remaining ~25 cm of the shoot in order to ensure similar distances along the shoot between the remaining 10 leaves. By only using older and very similar leaves, we reduced the possible effects of age, nutritional value, leaf area, and vertical position. First we allowed each female to lay eggs for four days on the same plant (treatment: first release; Sv $N = 27$ plants; Sd $N = 28$ plants) and then measured the distances between clutches (distance type: old–old; Appendix A). In the second part of the experiment, which lasted three days (beetles removed for ~5 h), plants that had received eggs were randomly assigned to one of the two following treatments: experienced (Sv $N = 13$ plants; Sd $N = 6$ plants), where we released the same female again onto the respective plant, or naive (Sv $N = 13$ plants; Sd $N = 14$ plants), where we released new ovipositing females that had not yet laid any eggs on any of the plants. We again measured all distances between these new clutches (new–new) and between the new clutches and the old clutches (old–new). Clutch location on the leaf, egg number, leaf area, and distances between clutches were obtained from photographs using the ImageJ software (Appendix A). By adding clutch–petiole distances and the distance between leaves with clutches (measured with a ruler along the shoot), we obtained all distances between all clutches on the 10 leaves on each plant for both *Salix* species.

Naturally, the distances between clutches decreased with increasing number of clutches on a plant (Appendix B: Figs. B1 and B2), and we standardized the distances by dividing each distance by the number of clutches on each plant. Based on the proportion of used and not-used leaves for oviposition, we checked if different distances were only due to females ovipositing on leaves with no eggs. Based on the proportion of clutches closer to the leaf petiole than the previous clutch(es) on that leaf, we investigated whether females facilitate larvae reaching new leaves if the leaf is already occupied.

Statistical analyses

The count data from Experiment 1 were analyzed with generalized linear mixed models (GLMMs) with Poisson distributions. The clutch–distance data from Experiment 2 were impossible to model, so we compared mean distances between clutches using the Kolmogorov–Smirnov (KS) test without adjusting the significance levels. A GLMM with a binomial distribution was used to test if females in the second part of Experiment 2 preferred to oviposit on leaves with eggs and if this proportion is affected by the plant species and/or the treatment. By using the leaves that received eggs in the first part and second part of Experiment 2 and using a similar model, we tested if females preferred to oviposit closer to the leaf petiole compared to the old clutches. All analyses were performed using R (R Core Team 2014). For a description of ImageJ, the models, the R packages, and the reasoning for not adjusting the KS test, see Appendix A.

RESULTS

Experiment 1: Influence of host occupancy level on host acceptance

Plant species and treatment affected the number of eggs laid (Table 1). As expected, between two and five times more eggs were laid on the more suitable Sv than on Sd. In line with our hypotheses, most eggs were laid on Sv in the mixed hosts treatment (Fig. 1a) showing that no and overwhelming number of conspecifics result in low host acceptance. The host-specific clutch sizes followed this general pattern (Table 1, Fig. 1b).

Experiment 2: Influence of cues and memory on clutch distance modulation

If an experienced female was released on Sv (experienced), it established clutches at greater distances from each other than on the previous visit, and these new clutches were even farther away from the old clutches (old–old < new–new < old–new; Fig. 2a). Naive females (naive) did not increase the distances between their clutches compared to the conspecific clutch distances but did lay their clutches farther away from the old clutches (old–old = new–new < old–new), although not as far as experienced females. On Sd, naive females increased new–new compared to old–old distances, and experienced females showed the largest increases in

TABLE 1. Analysis of variance (ANOVA) tables from generalized linear mixed models investigating the oviposition behavior of the blue willow leaf beetle *Phratorea vulgatissima*.

Question and explanatory variables	χ^2	df	P
Different number of eggs on plant			
Intercept	1820.24	1	<0.001
PS	60.42	1	<0.001
T	59.90	2	<0.001
PS × T	8.47	2	0.01
Different clutch sizes on plant			
Intercept	580.70	1	<0.001
PS	8.19	1	<0.01
T	6.91	2	0.03
PS × T	7.12	2	0.02
Preferred ovipositing on leaves with eggs			
Intercept	20.46	1	<0.001
PS	2.00	1	0.15
TR	0.04	1	0.83
PS × TR	2.04	1	0.15
Clutch closer to petiole than previous clutch			
Intercept	1.65	1	0.19
PS	1.25	1	0.26
TR	0.91	1	0.33
PS × TR	0.00	1	0.94

Notes: Different number of eggs on plant and clutch sizes on plant were addressed in the first experiment; preferred ovipositing on leaves with eggs and clutch closer to petiole than previous clutch were addressed in the second experiment. Nonsignificant values (italicized) were removed stepwise from the final model starting from the bottom row. Variables included plant species (*Salix viminalis*, *S. dasyclados*; PS); host treatment (same host, new hosts, mixed hosts; T), and release treatment (first release, naive, experienced; TR).

distance. There was great variation in the number of clutches and therefore the number of obtained distances (Appendix A: Table A2) and in the length of these distances (Appendix B: Fig. B1). To separate the effect of generally greater distances on *Sd* due to greater leaf area (Appendix B: Fig. B2) and/or due to increasing distances on an unsuitable genotype, we further standardized the distances based on the total leaf area (Fig. 2b). Except between the old–new distances of the experienced females, no differences between *Salix* species were found. We also examined the proportions of released females ovipositing on leaves with eggs and the proportion of clutches closer to the petiole than the previous clutch (Table 1). Regardless of plant species or the identity of established clutches, unoccupied leaves were used around twice as frequently (77 times; clutches from all plants) as occupied leaves (29 times; clutches from all plants, including multiple choices), but there was no significant difference in the number of times the females laid eggs closer to the petiole (18 times) compared to the previous clutch on a given leaf (11 times).

DISCUSSION

In Experiment 1, females laid more eggs on plants with intermediate occupancy than plants that had been

visited by many females or plants that were only visited by one female. We interpret this behavior as indicating that females take into account trade-offs between the advantages and disadvantages of group living during oviposition. Although a similar behavior has been shown for a root herbivore (Robert et al. 2012) and a leaf beetle (Meiners et al. 2005), we are aware of no previous studies demonstrating such fine-tuned behavior with respect to the fitness-relevant trait of oviposition (Navasero and Ramaswamy 1993, Vasconcellos-Neto and Monteiro 1993, Raitanen et al. 2013). The oviposition rate of these beetles is relatively stable over time, a good predictor of fitness, and determined by plant quality (Lehrman et al. 2012). That this gregarious species shows a lower oviposition rate if a female is alone on the plant could originate from the lack of conspecifics confirming its own decision. This behavior, and the behavior to avoid host plants that are too crowded, might have fitness consequences, because females in such situations would continue to search for better resource patches. We did not intend to directly observe switches to an unoccupied plant. But because host acceptance is plant-quality specific, and despite the

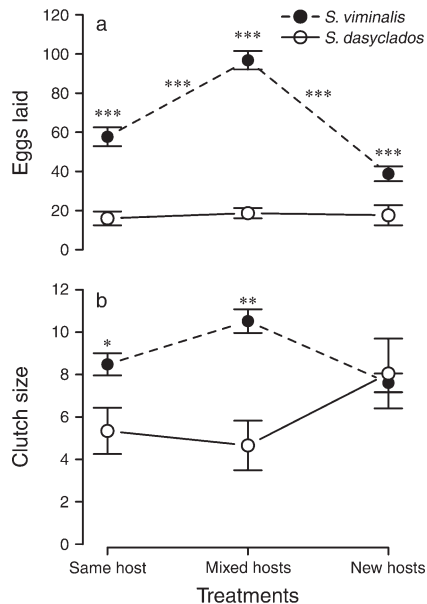


FIG. 1. (a) Number (mean ± SE) of blue willow leaf beetle (*Phratorea vulgatissima*) eggs laid and (b) clutch size on a plant after nine days of ovipositing on a suitable (*Salix viminalis*) or an unsuitable (*S. dasyclados*) host plant. Females were released daily onto the same plant (same hosts), in a rotational manner onto a new plant every day (new hosts), or in an intermediate release pattern (mixed hosts), resulting in the (a) highest host acceptance and (b) largest clutches if females encountered their own and conspecific cues on *S. viminalis*. Significant differences were determined via Tukey contrast.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

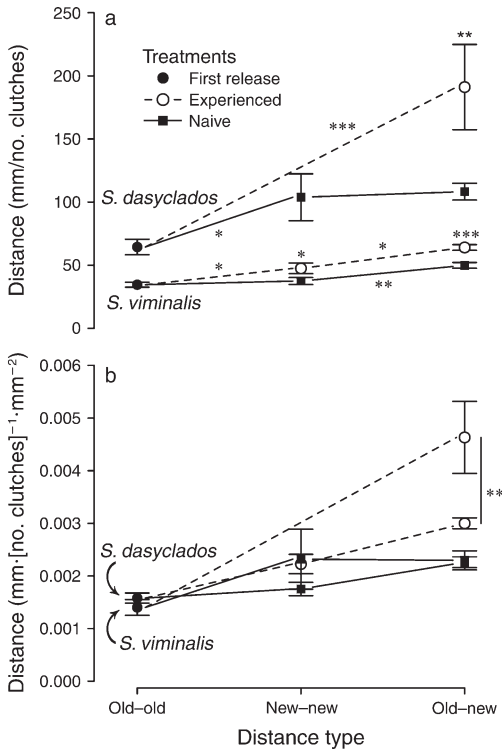


FIG. 2. Distances (mean \pm SE) between egg clutches of the leaf beetle (*P. vulgatissima*) on a shoot after (a) correcting for shoots with different clutch numbers and (b) additional correcting for *Salix* species with different total leaf areas; *S. dasyclados* has leaves twice as large as those of *S. viminalis*, and further standardizing of the distances revealed that generally larger distances are due to larger leaves, not to differences in host plant suitability. Largest clutch distances were found among new–new and old–new clutches in part two of the experiment for experienced females that were released on the same respective plants as in part one, and could rely on perceived cues and memory (compare to old–old clutches; old clutches are those laid in part one, new clutches were laid in part two). Naive females released in part two that had not encountered the respective plant before could only rely on cues. Significant differences were determined via a Kolmogorov-Smirnov test.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

fact that females might have left a plant earlier and generally laid fewer eggs out of awareness of alternative host plants, we interpret the observed pattern as finding a suitable level of aggregation that solves the trade-off between increasing offspring food competition and positive effects of group living.

How this species evaluates occupancy is not fully understood. Herbivory combined with beetle presence attracts other adults, suggesting that plant volatiles and pheromones are involved (Peacock et al. 2001), but other cues might also help determine occupancy (Fernandez and Hilker 2007, Dicke and Baldwin

2010). Early eggs probably contributed more to the final number of eggs on a plant than later eggs because females probably laid fewer eggs as they encountered more eggs on a plant over the course of the experiment. This reduced oviposition rate due to increasing egg number occurred in all three treatments and does not explain why the lowest egg numbers were found on plants visited by many females. The assumption that the lower rate is due to the number of conspecifics can, however, explain why the fewest eggs were found in the new hosts treatment (see Appendix B: Fig. B3). Here, the number of conspecifics increased rapidly and females probably responded with a stronger decrease in oviposition rate. The intermediate number of conspecifics resulting in the most eggs indicates that females were more sensitive to how many conspecifics had visited a plant than to the number of eggs on a plant. We also found that the level of aggregation did not differ on the unsuitable host. Here, acceptance was probably so low that striking a balance between competition and group advantages was irrelevant. We also saw that clutch size increased with host suitability. Females might have a physiological constraint and be unable to fully stop producing eggs on unsuitable hosts, resulting in a few small clutches, or because the same leaf area of hosts with different suitability will support different numbers of larvae, females might match clutch size to the nutritional value per leaf area.

In Experiment 2, we were interested in the beetle's cognitive capabilities and used the mean distance between clutches to describe the intra-plant clutch distribution. The largest clutch distances were found for experienced females relying on perceived cues and memory because they themselves established the previous clutches on a plant. This implies that they can memorize some spatial information about their previous oviposition and are able to minimize competition between sibling/non-sibling larvae. The distance modulations between their own clutches and in relation to conspecific clutches are very strong responses and are probably adaptive. They might represent a previously unknown behavioral component of insect oviposition that increases the chances of progeny survival. Such an increase in survival would be due to mechanisms that are relevant to aggregating organisms because the larvae from nearby clutches will form a group. The existence of such fine-tuned distance modulation extends the applicability of the term egg pooling/egg clumping/egg clustering that normally refers to eggs that have contact with at least one other conspecific egg. Arguments for the existence of such modulation should, therefore, be similar to those related to egg pooling and include host-plant nutrient distribution (Chew and Courtney 1991) and increased female fecundity (Courtney 1984). Although females also showed the often-observed behavior of frequently choosing unoccupied leaves (Whitham 1978), this was not the reason for the increased distances, because these choices were independent of

plant species and female experience. Previous studies have shown that intra-plant egg distribution varies on host plant species of different suitability (Silva and Furlong 2012), but we showed that leaf morphology in itself might also be important. Females increased the distances between clutches on a less suitable host plant due to differences in leaf area and not host suitability, as we did not find any differences between hosts after standardizing for total leaf area.

Females of the blue willow leaf beetle apparently not only use perceived cues during oviposition and anticipated future larval feeding, but also use some kind of memory to further improve their egg distribution. Learning and memory involve costs (Dukas 2008), but increased fitness due to optimized oviposition should be a strong evolutionary driver and should compensate for these. How these beetles acquire and use their memory in this process, however, remains unexplored. The method of using the final egg distribution as a more indirect proxy than classical movement observations appears to be novel. This method has the advantage of easily showing the existence of spatial memory (or a cognitive map), but the mechanism remains to be identified. Nevertheless, cognitive abilities are assumed to alter trophic interactions and population dynamics (Kondoh 2010), and their effect during oviposition should have major implications, especially for gregarious out-breaking species like *P. vulgatissima*. Both behaviors (finding a suitable level of aggregation and the optimized clutch distances) contribute to the mechanistic understanding of the spread of insect outbreaks.

ACKNOWLEDGMENTS

We thank for practical help Karin Eklund, Caroline Jöngren, Staffan Matzén, and Katharina Stephan; for statistical advice Mikael Andersson Franko; and for funding, the Swedish Energy Agency (Energimyndigheten), Future Forest, and the Swedish Research Council Formas. We also thank two anonymous reviewers, but especially the editor for valuable comments.

LITERATURE CITED

- Bergström, A., N. Janz, and S. Nylin. 2006. Putting more eggs in the best basket: clutch-size regulation in the comma butterfly. *Ecological Entomology* 31:255–260.
- Bitume, E., D. Bonte, O. Ronce, and F. Bach. 2013. Density and genetic relatedness increase dispersal distance in a subsocial organism. *Ecology Letters* 16:430–437.
- Björkman, C., B. Bengtsson, and H. Häggström. 2000. Localized outbreak of a willow leaf beetle: plant vigor or natural enemies? *Population Ecology* 42:91–96.
- Björkman, C., and K. Eklund. 2006. Factors affecting willow leaf beetles (*Phratora vulgatissima*) when selecting overwintering sites. *Agricultural and Forest Entomology* 8:97–101.
- Chew, F., and S. Courtney. 1991. Plant apparency and evolutionary escape from insect herbivory. *American Naturalist* 138:729–750.
- Clark, B., and S. Faeth. 1997. The consequences of larval aggregation in the butterfly *Chlosyne lacinia*. *Ecological Entomology* 22:408–415.
- Collett, M., L. Chittka, and T. Collett. 2013. Spatial memory in insect navigation. *Current Biology* 23:789–800.
- Courtney, S. 1984. The evolution of egg clustering by butterflies and other insects. *American Naturalist* 123:276–281.
- Dalin, P., O. Kindvall, and C. Björkman. 2009. Reduced population control of an insect pest in managed willow monocultures. *PLoS ONE* 4:e5487.
- Denno, R., and B. Benrey. 1997. Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. *Ecological Entomology* 22:133–141.
- Dicke, M., and I. T. Baldwin. 2010. The evolutionary context for herbivore-induced plant volatiles: beyond the “cry for help”. *Trends in Plant Science* 15:167–175.
- Doligez, B., E. Danchin, and J. Clobert. 2002. Public information and breeding habitat selection in a wild bird population. *Science* 297:1168–1170.
- Dukas, R. 2008. Evolutionary biology of insect learning. *Annual Review of Entomology* 53:145–160.
- Faraji, F., A. Janssen, and P. Van Rijn. 2000. Kin recognition by the predatory mite *Iphiseius degenerans*: discrimination among own, conspecific, and heterospecific eggs. *Ecological Entomology* 25:147–155.
- Fernandez, P., and M. Hilker. 2007. Host plant location by Chrysomelidae. *Basic and Applied Ecology* 8:97–116.
- Folmer, E., H. Olf, and T. Piersma. 2012. The spatial distribution of flocking foragers: disentangling the effects of food availability, interference and conspecific attraction by means of spatial autoregressive modeling. *Oikos* 121:551–561.
- Fretwell, S. D. 1969. On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheoretica* 19(1):16–36.
- Godfray, H. C. J. 1986. Clutch size in a leaf-mining fly (*Pegomya nigritarsis*: Anthomiidae). *Ecological Entomology* 11:75–81.
- Godfray, H. C. J., L. Partridge, and P. H. Harvey. 1991. Clutch size. *Annual Review of Ecology and Systematics* 22:409–429.
- Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. 2010. A meta-analysis of preference-performance relationships in phytophagous insects. *Ecology Letters* 13:383–393.
- Heisswolf, A., H. Poethke, and E. Obermaier. 2006. Multi-trophic influences on egg distribution in a specialized leaf beetle at multiple spatial scales. *Basic and Applied Ecology* 7: 565–576.
- Held, D. W., T. Eaton, and D. A. Potter. 2001. Potential for habituation to a neem-based feeding deterrent in Japanese beetles, *Popillia japonica*. *Entomologia Experimentalis et Applicata* 101:25–32.
- Higashiura, Y. 1989. Survival of eggs in the gypsy moth *Lymantria dispar*. II. Oviposition site selection in changing environments. *Journal of Animal Ecology* 58:413–426.
- Hunter, A. F. 2000. Gregariousness and repellent defences in the survival of phytophagous insects. *Oikos* 91:213–224.
- Jaenike, J. 1978. On optimal oviposition behavior in phytophagous insects. *Theoretical Population Biology* 14:350–356.
- Joos, B., T. Casey, T. Fitzgerald, and W. Buttemer. 1988. Roles of the tent in behavioral thermoregulation of eastern tent caterpillars. *Ecology* 69:2004–2011.
- Kendall, D., C. Wiltshire, and M. Butcher. 1996. Phenology and population dynamics of willow beetles (Coleoptera; Chrysomelidae) in short-rotation coppiced willows at Long Ashton. ETSU (DTI) Biofuels Study B/M4/00487/14/REP. IACR Long Ashton Research Station, Long Ashton, Bristol, UK.
- Kondoh, M. 2010. Linking learning adaptation to trophic interactions: a brain size-based approach. *Functional Ecology* 24:35–43.
- Lack, D. 1947. The significance of clutch size. *Ibis* 89:302–352.
- Le Goff, G., A. C. Mailloux, C. Detrain, J. L. Deneubourg, G. Clotuche, and T. Hance. 2009. Spatial distribution and inbreeding in *Tetranychus urticae*. *Comptes Rendus Biologies* 332:927–933.

- Lehrman, A., M. Torp, J. A. Stenberg, R. Julkunen-Tiitto, and C. Björkman. 2012. Estimating direct resistance in willows against a major insect pest, *Phratora vulgatissima*, by comparing life history traits. *Entomologia Experimentalis et Applicata* 144:93–100.
- Loeb, M., L. Diener, and D. Pfennig. 2000. Egg-dumping lace bugs preferentially oviposit with kin. *Animal Behaviour* 59: 379–383.
- Matsumoto, K. 1990. Population dynamics of *Luehdorfia japonica* Leech (Lepidoptera: Papilionidae). II. Patterns of mortality in immatures in relation to egg cluster size. *Researches on Population Ecology* 32:173–188.
- Meiners, T., N. K. Hacker, P. Anderson, and M. Hilker. 2005. Response of the elm leaf beetle to host plants induced by oviposition and feeding: the infestation rate matters. *Entomologia Experimentalis et Applicata* 115:171–177.
- Meiners, T., and E. Obermaier. 2004. Hide and seek on two spatial scales—vegetation structure effects herbivore oviposition and egg parasitism. *Basic and Applied Ecology* 94:87–94.
- Mitchell, R. 1975. The evolution of oviposition tactics in the bean weevil, *Callosobruchus maculatus* (F.). *Ecology* 56:696–702.
- Navasero, R., and S. Ramaswamy. 1993. Influence of plant age, water stress, larval damage, and presence of conspecific eggs on oviposition by *Heliothis virescens* (F.) on cotton. *Journal of Applied Entomology* 115:97–106.
- Obermaier, E., and H. Zwölfer. 1999. Plant quality or quantity? Host exploitation strategies in three Chrysomelidae species associated with Asteraceae host plants. *Entomologia Experimentalis et Applicata* 92:165–177.
- Peacock, L., and S. Herrick. 2000. Responses of the willow beetle *Phratora vulgatissima* to genetically and spatially diverse *Salix* spp. plantations. *Journal of Applied Ecology* 37:821–831.
- Peacock, L., M. Lewis, and S. Herrick. 2001. Factors influencing the aggregative response of the blue willow beetle, *Phratora vulgatissima*. *Entomologia Experimentalis et Applicata* 98:195–201.
- R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Raitanen, J., J. T. Forsman, S. M. Kivelä, M. I. Mäenpää, and P. Välimäki. 2013. Attraction to conspecific eggs may guide oviposition site selection in a solitary insect. *Behavioral Ecology* 25:110–116.
- Rausher, M. D. 1983. Conditioning and genetic variation as causes of individual variation in the oviposition behaviour of the tortoise beetle, *Deloyala guttata*. *Animal Behaviour* 31: 743–747.
- Robert, C., M. Erb, B. E. Hibbard, B. W. French, C. Zwahlen, and T. C. J. Turlings. 2012. A specialist root herbivore reduces plant resistance and uses an induced plant volatile to aggregate in a density-dependent manner. *Functional Ecology* 26:1429–1440.
- Siemens, D., and C. Johnson. 1992. Density-dependent egg parasitism as a determinant of clutch size in bruchid beetles (Coleoptera: Bruchidae). *Environmental Entomology* 21: 610–619.
- Silva, R., and M. J. Furlong. 2012. Diamondback moth oviposition: effects of host plant and herbivory. *Entomologia Experimentalis et Applicata* 143:218–230.
- Srinivasan, M. V. 2010. Honey bees as a model for vision, perception, and cognition. *Annual Review of Entomology* 55:267–284.
- Stenberg, J. A., A. Lehrman, and C. Björkman. 2010. Uncoupling direct and indirect plant defences: novel opportunities for improving crop security in willow plantations. *Agriculture, Ecosystems and Environment* 139:528–533.
- Subinprasert, S., and B. W. Svensson. 1988. Effects of predation on clutch size and egg dispersion in the codling moth *Laspeyresia pomonella*. *Ecological Entomology* 13:87–94.
- Vasconcellos-Neto, J., and R. F. Monteiro. 1993. Inspection and evaluation of host plant by the butterfly *Mechanitis lysimnia* (Nymph., Ithomiinae) before laying eggs: a mechanism to reduce intraspecific competition. *Oecologia* 95:431–438.
- Whitham, T. 1978. Habitat selection by *Pemphigus* aphids in response to response limitation and competition. *Ecology* 59: 1164–1176.
- Wright, G., and F. Schiestl. 2009. The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Functional Ecology* 23: 841–851.
- Zink, A. G. 2003. Intraspecific brood parasitism as a conditional reproductive tactic in the treehopper *Publilia concava*. *Behavioral Ecology and Sociobiology* 54:406–415.

SUPPLEMENTAL MATERIAL

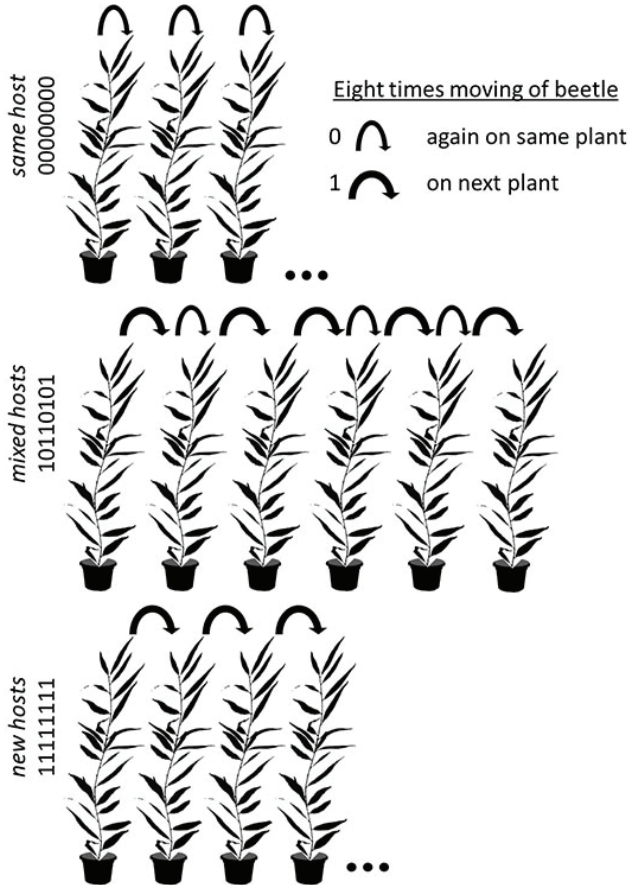
Ecological Archives

Appendices A and B are available online: <http://dx.doi.org/10.1890/14-1143.1.sm>

Jörg G. Stephan, Johan A. Stenberg, and Christer Björkman. 2015. How far away is the next basket of eggs? Spatial memory and perceived cues shape aggregation patterns in a leaf beetle. *Ecology* 96:908–914. <http://dx.doi.org/10.1890/14-1143.1>

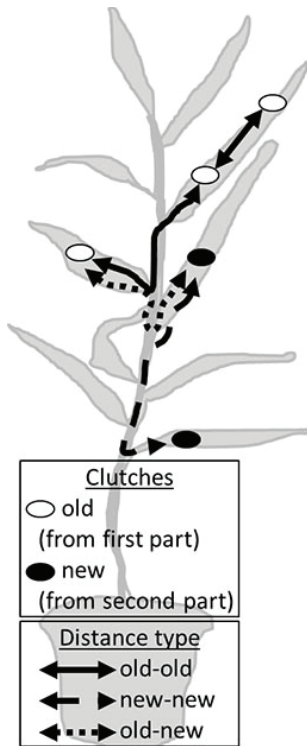
APPENDIX A. Experimental set ups, software, model description, and statistics.

Set up of Experiment 1:



All plants in the *mixed hosts* and *new hosts* treatments could be imagined in a circle, respectively. If a beetle died/escaped the plant was excluded and the next plant in the circle was used. Therefore all *mixed hosts* plants were visited by 5 different females (that encountered 6 different plants) and all *new hosts* plants by 8 different females (that encountered 9 different plants). By distributing the change to the next plant in the *mixed hosts* over time we attempted to account for the fact that beetles in the later part of the experiment encountered plants with more eggs (see Appendix B: Fig. B3). In the *same host* treatment only plants where females laid eggs until the 9th day were used; plants where females died/escaped were excluded.

Set up of Experiment 2:



Females were allowed to lay egg clutches (old) where after the distances between these clutches were measured. Then either the same female or a female from the rearing was released again and laid clutches (new) where after the distances between these new clutches (new-new) and their relation to the already established clutches (old-new) were measured.

Picture analysis with ImageJ:

Pictures of leaves on the shoots were taken with a Canon Digital IXUS 60 while holding a white sheet of paper behind each leaf. The sheet was equipped with a piece of red tape 50 mm in length. Pictures were analyzed using ImageJ (Schneider et al. 2012). For each picture, the scale was set by using the red tape as the internal standard (draw line on 50 mm stripe → Analyze → Set Scale). Distances between clutch edge and leaf petiole were then measured on pictures of the leaf using straight lines (draw line → Analyze → Measure), and eggs within clutches were counted using the Multi-Point Tool on pictures of each clutch. For the leaf area measurements, pictures of individual leaves taken before the experiment were used. Each leaf was roughly surrounded with the Polygon Selection tool followed by removal of the outside (Edit → Clear outside). They were converted to grayscale (Image → Type → 8-bit) and made binary (Process → Binary → Make Binary) leaving the leaf in black on a white background. After measuring (and setting the Set Measurements within the Result window to Area and Limit to threshold), individual leaf areas were obtained. All commands after the selection were combined into a macro and executed with a shortcut.

Statistical analysis with R:

For the linear mixed model (LMM) and the generalized linear mixed models (GLMM), we used the function *lmer* and *glmer* from the package *lme4* (Bates et al. 2012), and pair-wise comparisons were performed with the function *glht* from the package *multcomp* (Hothorn et al. 2008). Requirements for the LMM were checked visually (Zuur et al. 2009). The conditional and marginal pseudo R^2 values were calculated using the function *r.squaredGLMM* from the package *MuMIn* (Bartoń 2014). The analysis-of-variance tables were obtained with the function *Anova* from the package *car* (Fox and Weisberg 2011).

Model selection:

We started with full models including all interactions and used a backwards selection by removing non-significant interactions followed by non-significant main effects. The type-III analysis-of-variance tables with Wald chi-square tests were used. We included an information criterion (AIC) to show that the most parsimonious model was reached during the model selection, and the marginal R-squared ($R^2_{GLMM(m)}$) and conditional R-squared ($R^2_{GLMM(c)}$) were used to give an estimate for the absolute model fit and variance explained (Nakagawa and Schielzeth 2013) (Table A1).

Experiment 1:

The count data of the number of eggs laid on a plant and the clutch size were analyzed with a GLMM with Poisson distribution, a log link function, and each plant or each clutch nested within each plant as a Gaussian random factor for the number of eggs and the clutch sizes, respectively. Therefore, hierarchical data structures and possible model overdispersion were accounted for.

Experiment 2:

The clutch distance data were not possible to model because they were unbalanced, hierarchical (nested within each plant), not independent (each distance on each plant depended on the other distances), and were missing distance type–treatment combinations (e.g., no *new-new* × *first release*). The KS test normally also requires independent samples, but due to the lack of alternatives we decided to use this very conservative test. We did not adjust the significance levels because—contrary to many ecological studies—we had very large sample sizes that gave one mean value (Table A2). Most importantly, adjusting the significance level excludes the possibility of a Type I error but increases the likelihood of a Type II error (Moran 2003). Because the lack of overlapping standard errors indicated significant differences, we did not want to take the risk missing biologically relevant information. Nevertheless, correcting for multiple comparisons results in strong tendencies instead of significant comparisons (Table A3).

The proportions of leaves with and without eggs from the first female that were used by the second female on a plant were analyzed with a GLMM with a binomial distribution, a logit link function, and each observation as a Gaussian random factor. The proportion of these new clutches that were laid closer to the leaf petiole than the old clutches was analyzed with a GLMM with a binomial distribution, a logit link function, and each observation as a Gaussian random factor.

TABLE A1. Extended analysis-of-variance tables from generalized linear mixed models. Non-significant terms (italicized) were removed stepwise from the final model starting from the bottom row. PS = Plant Species (*Salix viminalis*, *Salix dasyclados*); T = Treatment (*same host*, *new hosts*, *mixed hosts*); TR = Treatment (*first release*, *naive*, *experienced*).

Experiment	Question	Explanatory Variables	X2	df	AIC	R ² GLMM(m)	R ² GLMM(e)	p value
1	Different number of eggs on plant	intercept	1820.24	1	423.55	0.84	0.84	<0.001
		PS	60.42	1	423.55	0.84	0.84	<0.001
		T	59.90	2	423.55	0.84	0.84	<0.001
		PS × T	8.47	2	423.55	0.84	0.84	0.01
1	Different clutch sizes on plant	intercept	580.70	1	1824.81	0.12	0.16	<0.001
		PS	8.19	1	1824.81	0.12	0.16	<0.01
		T	6.91	2	1824.81	0.12	0.16	0.03
		PS × T	7.12	2	1824.81	0.12	0.16	0.02
2	Preferred ovipositing on leaves with eggs	intercept	20.46	1	90.81	0.00	0.17	<0.001
		PS	2.00	1	90.65	0.04	0.19	0.15
		TR	0.04	1	92.61	0.04	0.19	0.83
		PS × TR	2.04	1	92.69	0.09	0.22	0.15
2	Clutch closer to petiole than previous clutch	intercept	1.65	1	34.74	0.00	0.01	0.19
		PS	1.25	1	35.54	0.06	0.06	0.26

		TR	0.91	1	36.57	0.10	0.10	0.33
		PS × TR	0.00	1	38.57	0.10	0.10	0.94

TABLE A2. Number of obtained distances on *S. viminalis* (Sv) and *S. dasyclados* (Sd) after the experiment. For example, ten clutches resulted in 45 interdependent distances that captured the clutch distribution of that female (distance number = (clutch number - 1) × clutch number / 2). (* only 11 of the 19 plants used received a maximum of one clutch and, therefore, there were no new-new distances).

Treatment	Part	Distance type	N
first release	1	old-old	Sv 180 Sd 52
naïve	2	new-new	Sv 68 Sd 10
		old-new	Sv 171 Sd 54
experienced	2	new-new	Sv 42 Sd 0*
		old-new	Sv 179 Sd 11

TABLE A3. *P* values and corrected *p* values between pair-wise comparisons of interest for *Salix viminalis* (Sv) and *Salix dasyclados* (Sd)

Pair-wise comparison		<i>p</i> value	Bonferroni-Holm corrected <i>p</i> value	
Sv	experienced × old-new	naïve × old-new	0.00000588	0.00003528
	naïve × new-new	naïve × old-new	0.001144	0.00572
	experienced × new-new	experienced × old-new	0.01828	0.07312
	experienced × new-new	first release × old-old	0.02911	0.08733
	experienced × new-new	naïve × new-new	0.04242	0.08484
	naïve × new-new	first release × old-old	0.1359	0.1359
Sd	first release × old-old	experienced × old-new	0.0006615	0.002646
	naïve × old-new	experienced × old-new	0.002818	0.008454
	first release × old-old	naïve × new-new	0.0265	0.053

<i>naive</i> × <i>old-new</i>	<i>naive</i> × <i>new-new</i>	0.9439	0.9439
-------------------------------	-------------------------------	--------	--------

LITERATURE CITED

- Bartoń, K. 2014. MuMIn: Multi-model inference. R package version 1.10.0. Retrieved May 14, 2014, from <http://cran.r-project.org/package=MuMIn>.
- Bates, D., M. Maechler, and B. Bolker. 2012. *lme4: Linear mixed-effects models using Eigen and Eigen++*. R package version 1.1-14.1.
- Fox, J., and S. Weisberg. 2011. *An {R} Companion to Applied Regression*. Sage Publications.
- Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50:346–363.
- Moran, M. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* 2:403–405.
- Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution* 4:133–142.
- Schneider, C., W. Rasband, and K. W. Eliceiri. 2012. NIH image to imageJ: 25 years of image analysis. *Nature methods* 9:671–675.
- Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. *Mixed effects models and extensions in ecology with R*. Genetics. Springer Verlag.
-

Jörg G. Stephan, Johan A. Stenberg, and Christer Björkman. 2015. How far away is the next basket of eggs? Spatial memory and perceived cues shape aggregation patterns in a leaf beetle. *Ecology* 96:908–914. <http://dx.doi.org/10.1890/14-1143.1>

APPENDIX B. Decreasing distance with increasing number of clutches and believed oviposition pattern.

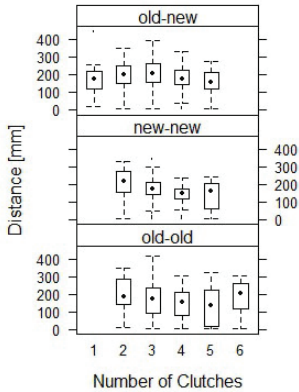


FIG. B1. Box plot of intra-plant distances between clutches depending on the number of clutches on a plant. Irrespective of plant genotype, the mean distance between clutches was dependent on the number of clutches on one shoot (Linear mixed model with plant as random effect: clutch number: Chi-square = 14.2, $df = 1$, $p < 0.001$; plant species: Chi-square = 1.1, $df = 1$, $p = 0.29$; clutch number \times plant species: Chi-square = 0.7, $df = 1$, $p = 0.38$).

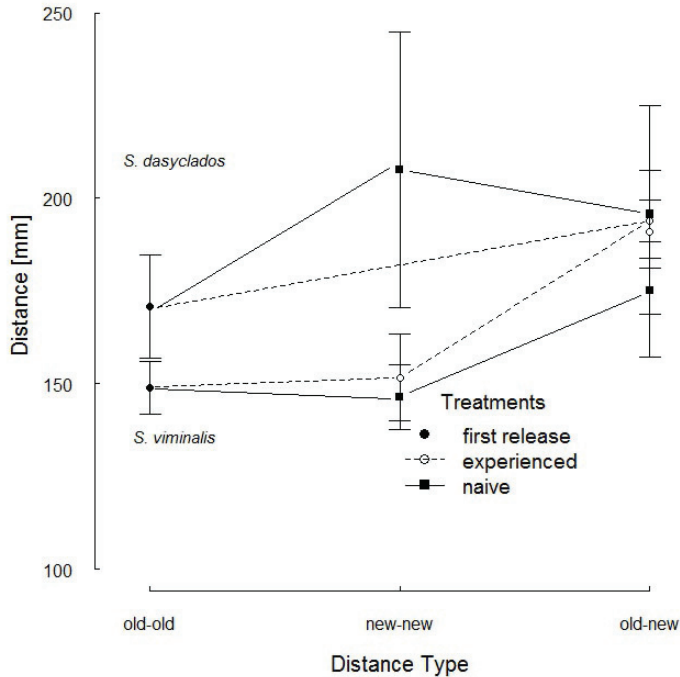


FIG. B2. Original distances between clutches from all plants. These distances were standardized by the number of clutches on a plant and further divided by the total plant leaf area of each plant (Mean, SD, Min, Max leaf area (mm²) of one leaf: *S. viminialis*: 2186, 450, 1047, 3469; *S. dasyclados*: 4880, 1591, 1518, 10260).

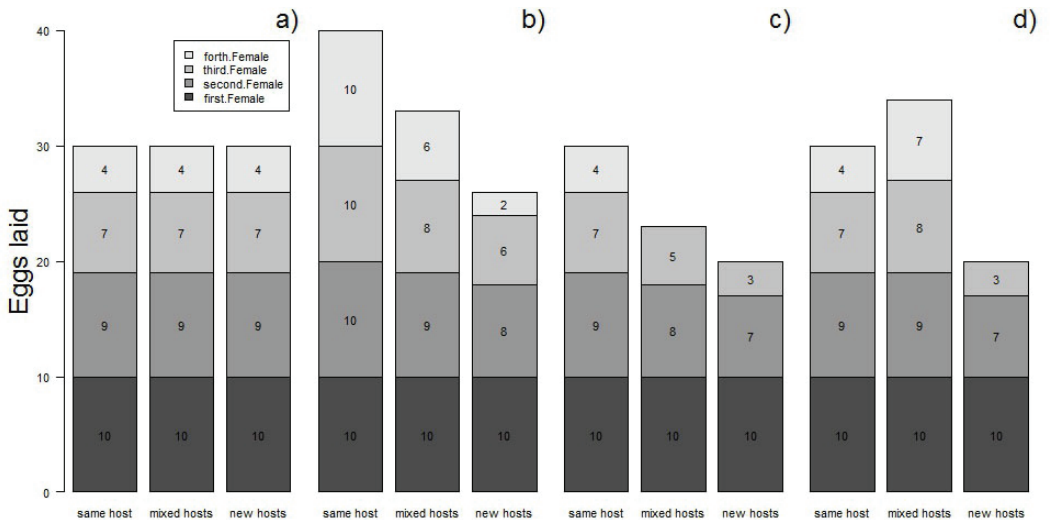


FIG. B3. Example calculation of believed oviposition patterns for each female during the experiment with four females (all spent the same amount of time on a plant, and fewer eggs implies a lower oviposition rate (OR)). The first females laid the same number of eggs, but subsequent females laid fewer. (a) The OR decreases with increasing number of eggs on a plant. (b) The OR decreases with number of conspecifics visiting a plant; the number of conspecifics increases faster on the *new host* than on the *mixed hosts*

and, therefore, the OR decrease was greater in the *new hosts* treatment. (c) The cumulative OR decreases due to number of eggs and due to number of conspecifics. (d) The observed highest egg numbers in the *mixed hosts* treatment must originate from a higher OR although the egg number on a plant was increasing, otherwise *mixed hosts* would have received as many eggs as the *same host* treatment. Therefore, females in this treatment must have ranked the preferred intermediate conspecific density higher than the higher number of eggs on a plant.

Habitat quality and predator hunting mode interactively affect attack-abatement patterns of predation risk

Jörg G. Stephan^{1*}, Matthew Low^{1#}, Johan A. Stenberg², and Christer Björkman¹

¹ Department of Ecology, Swedish University of Agricultural Sciences, PO Box 7044, SE-75007 Uppsala, Sweden

² Department of Plant Protection Biology, Swedish University of Agricultural Sciences, PO Box 102, SE-23053 Alnarp, Sweden

*Corresponding author e-mail: jorg.stephan@slu.se

#share first authorship

Abstract

Group formation reduces individual predation risk when the proportions of prey taken per predator encounter declines faster than the increase in group-encounter rate (attack-abatement). Despite attack-abatement being an important component of group-formation ecology, several key aspects have not been empirically studied: i.e. interactions with the hunting mode of the predator and how these relationships are modified by local habitat quality. In 79 cage trials we examined individual egg predation risk in different-sized egg clutches from the blue willow beetle *Phratora vulgatissima* for two predators with different hunting modes (consumption of full-group [*Orthotylus marginalis*] versus part-group [*Anthocoris nemorum*]). Because these predators also take nutrients from plant sap, we could examine how the quality of alternative food sources (high versus low-quality host plant sap) influenced attack-abatement patterns in the presence of different hunting strategies. For the *O. marginalis* predator, individual egg predation risk was largely independent of group size. For *A. nemorum*, egg predation risk clearly declined with increasing group size. However, independently of groups becoming larger, approximately one third of the grouping benefit is lost due to increase in detectability. There were clear differences in attack-abatement patterns between plants with high versus low-quality sap. When *O. marginalis* was the predator, there was no clear change in attack-abatement in relation to plant quality. However, for *A. nemorum* there was a clear reduction in overall predation risk and a stronger attack-abatement pattern with increasing group size when plant-sap quality increased. This may imply that the relative benefits of prey grouping behaviour for any species could be expected to show diurnal or seasonal changes as other aspects of resource/habitat quality change for the focal predator. Modulation of attack-abatement by bottom-up effects such as plant-based food resources is yet to be incorporated into general theory, despite the ubiquity of omnivorous predators and with omnivory being important for shaping food webs, ecosystem functions and in biological control. Thus, ongoing refinement of attack-abatement theory by focusing on bottom-up versus top-down processes, often from a tritrophic interaction perspective, could have significant impacts on many important contemporary fields of study.

Keywords: aggregation, selfish herd, clutch size, habitat quality, avoidance, conspicuousness, dilution, omnivore, prey detection, searching strategy, tritrophic interaction, group structure

Introduction

Prey species have evolved many behaviours to reduce predation risk (Hendrichs *et al.*, 1991; Cocroft, 1999), with one of the most important being group living (Ebensperger, 2001; Krause & Ruxton, 2002; Pollard & Blumstein, 2008). A primary benefit of prey grouping behaviour results from group-size-related changes between the predator encounter rate and the proportion of the group preyed upon during each encounter: so called ‘attack-abatement’ (Turner & Pitcher, 1986). This occurs because the proportion of prey taken per encounter (a.k.a. numerical ‘dilution’ (Turner & Pitcher, 1986; Wrona & Dixon,

1991); in contrast to more unspecific use of ‘dilution’) generally declines at a rate faster than the corresponding increase in encounters related to group size (Fig. 1, Appendix A, (Turner & Pitcher, 1986; Inman & Krebs, 1987)).

Despite the central role attack-abatement has in explaining the advantages of prey group living (Krause & Ruxton, 2002; Davies *et al.*, 2012), few studies have successfully disentangled the relative contribution of encounter and dilution (but see (Foster & Treherne, 1981; Wrona & Dixon, 1991)) to reducing individual predation risk. This is because group-size-related effects such as predator confusion (Schradin, 2000),

alarm-signalling (Maschwitz, 1966), group defence (Cocroft, 1999), evasion (Weihs & Webb, 1984), vigilance (Roberts, 1996), disaggregation (Creel & Winnie, 2005) and group structure (Hamilton, 1971) including predator detection and information spread (Bednekoff & Lima, 1998) may co-vary with attack-abatement measures. In addition, studies of attack-abatement have not quantified how other processes potentially important in influencing predator or prey behaviour (e.g. bottom-up effects relating to resource quality and distribution and structural differences of the foraging area) may interact with these top-down group-formation benefits. Thus while it is generally acknowledged that these interactions are likely to influence prey distribution (Jensen & Larsson, 2002), there are currently no empirical studies demonstrating interactions between attack-abatement, predator behaviour and local habitat or resource quality, limiting theory development of group-formation ecology.

Although the attack-abatement model is often illustrated using a single idealized prey species under the full range of possible predatory conditions of encounter and dilution (Turner & Pitcher, 1986; Inman & Krebs, 1987), empirical support comes instead from comparisons of different prey species operating under specific fixed predator conditions - i.e. rather than varying the predator conditions for a specific prey, support comes from combining evidence from different predator-prey systems each with its own fixed encounter and dilution rates. In such cases the proportion of prey taken universally declines with increasing group size (Calvert *et al.*, 1979; Foster & Treherne, 1981; Wrona & Dixon, 1991; Uetz & Hieber, 1994). This is because the dilution effect in these studies is always complete (e.g. (Foster & Treherne, 1981)) or partial (e.g. (Wrona & Dixon, 1991)), meaning that the relationship between group size and predation risk under some conditions predicted by theory have never been tested - i.e. when the dilution effect is zero. Zero dilution occurs if all encountered prey are consumed, which depends on the predator hunting mode (Schmitz, 2007) and the response of the prey (e.g. if they are unable to escape). Thus it remains to be empirically confirmed how the relative benefits of grouping change as different predator hunting modes alter the encounter and dilution effects for a single prey species at both extremes of the range of dilution values within an attack-abatement context.

Determining the extent to which attack-abatement contributes to reducing individual predation risk can be difficult because it requires controlling for group-size-related effects that may co-vary with attack-abatement measures (e.g. predator confusion, communal defence, vigilance and group structure). One solution has been

to study sessile organisms (Wrona & Dixon, 1991); however, these may still include group structure effects if individuals display non-random settlement. To account for these issues, we used an experimental approach to study egg predation risk related to clutch (i.e. group) size in the blue willow beetle *Phratora vulgatissima* (Coleoptera: Chrysomelidae; hereafter: leaf beetle), because this well-studied system has characteristics that are ideal for studying attack-abatement. First, eggs are grouped into clutches that naturally vary in size from 1-50 that can be easily manipulated into specific group sizes with little variation in egg quality. Second, eggs are sessile and do not display any confounding anti-predator behaviours. Also, because egg clutches come from a single female and can be assumed to have an equal fitness value, there are no group structure effects complicating dilution calculations (e.g. Hamilton's selfish herd (Hamilton, 1971)). Third, the two primary natural predators of the leaf beetle's eggs have different hunting behaviours that correspond to the extremes of the dilution effect range: *Anthocoris nemorum* takes a certain number of eggs per attack meaning the proportion of prey taken is inversely related to group size, and *Orthotylus marginalis* consumes all eggs, meaning that the proportion of prey taken is constant regardless of group size. Thus by comparing egg survival relative to group size for these two active-hunting predators (Miller *et al.*, 2014), the attack-abatement theory can be empirically tested for the first time for both extremes of dilution effects within the same prey species. Finally, bottom-up (Godfray, 1986; Kagata & Ohgushi, 2002) and top-down processes (Subinprasert & Svensson, 1988) are important clutch-size determinants for herbivorous insects. In this system, the leaf beetle and the omnivorous egg predator *A. nemorum* are clearly influenced by the sap quality of different willow *Salix* genotypes (Stenberg *et al.*, 2010, 2011), with this having potential consequences for predator behaviour (as predators consume both leaf beetle eggs and plant sap). In addition, plant genotypes may differ with respect to plant architecture that could influence foraging behaviour of both predators because they are both omnivores (Gingras *et al.*, 2008). Thus, by examining how attack-abatement relationships change with respect to plant genotype, we can examine for the first time how bottom-up processes may interact with top-down attack-abatement effects.

We experimentally manipulated clutch sizes in the leaf beetle in the presence of two different predation strategies on two different willow species with two genotypes each. From this we asked the following questions based on the perspective of female beetles that lay many small groups, several mid-sized groups or one large group on each plant individual. First, does

egg predation risk relative to group size follow expectations from attack-abatement theory as the dilution effect varies from being complete (*A. nemorum*) to zero (*O. marginalis*) for a single prey species? Theory predicts that predation risk should be negatively related to group size in the presence of *A. nemorum* and independent for *O. marginalis* (Turner & Pitcher, 1986; Inman & Krebs, 1987). Second, to what extent does group size influence the group encounter rate for an egg predator, and is the effect similar between group size categories? If the encounter effect is complete and not related to group size (e.g. (Foster & Treherne, 1981)), then we expect the number of eggs taken per clutch by *A. nemorum* to be similar for all group sizes, resulting in a 9-fold survival advantage for eggs in the largest group compared to the smallest. If more eggs are taken from larger groups, this indicates a higher probability of group encounters as group size increases (i.e. a reduction of the encounter effect from complete to partial due to higher conspicuousness). Finally we investigate how the patterns of egg predation risk relative to group size vary on host plants differing in food quality and architecture. Omnivorous predators may alter their predatory behaviour relative to how well their plant-based dietary needs are satisfied (Vasseur & Fox, 2011) or due to the structure of the foraging area (Grevstad & Klepetka, 1992). Although we do not empirically separate changes in the foraging behaviour due to plant quality or due to plant architecture this would be the first evidence for interactions between bottom-up effects (host plants) on the predator and attack-abatement patterns within a tritrophic system.

Material and Methods

Study species

The adults and larvae of the blue willow beetle (*Phratora vulgatissima*) skeletonize the leaves of their host plants. This pest species is the most important specialist herbivore on willow (*Salix* spp.) in Europe, and it frequently outbreaks in plantations and in natural willow stands (Dalin *et al.*, 2009). Adults emerge in April after overwintering, mate after feeding for two weeks, and lay hundreds of eggs in clutches of 1–50 eggs on the underside of leaves. *Orthotylyus marginalis* (Heteroptera: Miridae; dark green apple capsid) is important in regulating leaf beetle population dynamics and is, together with *Anthocoris nemorum* (Heteroptera: Anthocoridae; common flower bug), the most common natural enemies of the leaf beetle in willow plantations (Björkman *et al.*, 2003). Although *A. nemorum* and *O. marginalis* are generally regarded as predators of the leaf beetle's eggs, both are omnivorous and also require host plant sap as part of

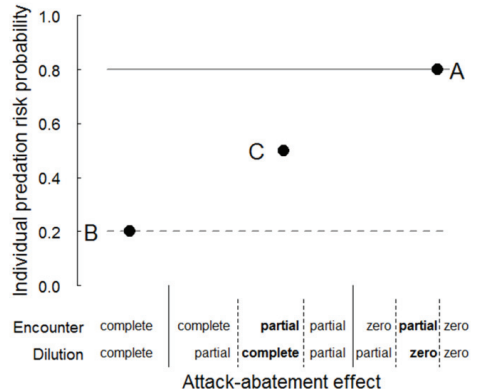


Figure 1: Individual predation risk probability relative to group size is determined by the multiplicative combination of the encounter and dilution effects relative to a solitary animal (or the smallest group size). In this example a solitary animal with predation risk ‘A’ joins a group of size N. If the group encounter rate is the same for all groups (i.e. complete encounter effect) and the proportion of prey taken per group is 1/N (i.e. complete dilution effect), then group formation results in an N-fold reduction in predation risk (‘B’). If either of the effects is zero (see also Case I and II in (Turner and Pitcher 1986)), predation risk does not change with group size (‘A’). If the predation risk of the smallest group is known, then observed predation risk of any group size can be compared relative to A (no attack-abatement) and B (full attack-abatement) that set the limits of grouping benefits (grey lines). Thus the group formation benefit of an observed ‘C’ can be calculated in absolute terms (A-C) or in terms relative to the potential for full attack-abatement (A-C / A-B). In our study (bold text) the dilution effect is either complete (*A. nemorum* consuming a constant number of eggs) or zero (*O. marginalis* consumes all eggs once encountered) and we can therefore estimate the weakening of the encounter effect for *A. nemorum* due to increased conspicuousness of larger clutches. If $A-C / A-B < 1$ this indicates encounter rates increasing with group size, with this ratio being an estimate of how much the encounter rate changes with group size.

their diet (Lehman, 1932). *A. nemorum* and *O. marginalis* have distinctly different egg hunting modes (Björkman *et al.*, 2003) and habitat domains (J. G. Stephan *et al.*, unpublished data) on willow shoots. *A. nemorum* preferentially forages in the upper part of the plant and displays an ‘eat and run’ predator feeding strategy where it only takes a certain number of eggs from a clutch before seeking out plant-based nutrients. The less mobile *O. marginalis* preferentially forages in the lower part of the plant and has a ‘find and stay’ predation strategy, where it consumes all eggs in a clutch before moving on. Thus, from attack-abatement theory, increasing group size should benefit the leaf beetle in the presence of *A. nemorum* (assuming the encounter rate does not increase in direct proportion to group size) but not in the presence of *O. marginalis*.

Different *Salix* genotypes vary in their quality as host plants for leaf beetles (Lehrman *et al.*, 2012) and their omnivorous predators (Stenberg *et al.*, 2010, 2011). Leaf beetles have a much higher fecundity on genotypes of *S. viminalis* (78183, 78021) than *S. dasyclados* (Loden, Gudrun). The predator *A. nemorum* has a higher fecundity on *S. dasyclados* over *S. viminalis* in the absence of leaf beetle prey; however this difference in host plant quality disappears when leaf beetle eggs are present (Stenberg *et al.*, 2011). This suggests that *A. nemorum* has less need for leaf beetle eggs on *S. dasyclados* with the host plant potentially influencing egg predation rates and attack-abatement patterns. Both plant species also differed in leaf morphology and plant architecture, with *S. dasyclados* ('simpler' architecture) having around half as many leaves that are approximately twice as large as *S. viminalis* ('complex' architecture). Thus, the predator search areas on each plant was similar (comparable cumulative leaf areas; same shoot height), but with different plant structures that conceivably could influence foraging behaviour (Gingras *et al.*, 2008).

Attack-abatement experiments

We measured the relationship between group size and egg predation risk during 79 replicated cage trials. For each trial, we took leaves with egg clutches from the rearing cage (see below), and using forceps under microscopy created fixed clutch sizes of 5, 15, or 45 eggs; this does not affect the viability of the eggs (J. G. Stephan, unpublished data). Each trial consisted of a *Salix* shoot that was divided into 13 equal-sized segments along its length, with several leaves in each segment. Each segment randomly received one clutch by pinning the leaf with the clutch onto the underside of one of the leaves in the segment (Appendix B). The total number of eggs on each plant and number of eggs in each size class were the same (each plant received 9 x 5, 3 x 15 and 1 x 45 egg-clutches); these were typical of egg numbers seen during outbreak years. Egg quality was assumed to be similar for all clutches because variation in the nutritional value of each egg was minimal and independent of the feeding source of the female (mean (mg/g) \pm SE: carbon: 492.9 \pm 8.4, nitrogen: 85.2 \pm 1.2; (Björkman *et al.*, 2011)), with no systematic differences in egg size within and between clutches (J. Stephan, unpublished data). The shoots were placed in cylindrical transparent plastic cages (height 70 cm, diameter 30 cm) covered with a net to allow air convection. In each cage, three *A. nemorum* individuals (43 experiments) or three *O. marginalis* individuals (36 experiments) were released for 72 hours. After this time, the numbers of empty eggshells were counted on each shoot and summed for each group size category (i.e. number preyed upon per 45

eggs). With this experimental design we ensured that: (i) the behaviour of both predators can be compared because we excluded the possibility of different responses to different prey densities (Wiedenmann & O'Neil, 1992), (ii) predator satiation will not come into play or will at least be the same on each clutch size class (here we also assumed that increased energy demand during searching for more scattered clutches is minimal and does not increase the satiation point), and (iii) changes in intraspecific interference due to different prey densities (Abrams & Ginzburg, 2000) were excluded.

For each predator we compared the relationship between group size and egg predation risk using two *Salix* host plant species known to differ in plant-nutrient quality for the omnivorous predators (i.e. *S. viminalis* genotypes '78021' & '78183' [low sap quality] and *S. dasyclados* genotypes 'Gudrun' & 'Loden' [high sap quality]; (Stenberg *et al.*, 2010)). For the 43 experiments with *A. nemorum*, 22 were on *S. viminalis* and 21 on *S. dasyclados*; for the 36 experiments with *O. marginalis*, 18 were on each *Salix* species.

Growth of plant material, insect maintenance, and all trials took place in a greenhouse (23°C, relative humidity 80%, 18:6 hr light:dark cycle). All insects were collected from natural populations in the Uppsala region of Sweden (59.85 N, 17.64 E). The leaf beetles were allowed to feed in rearing cages on *S. viminalis* genotype 78183 until reproduction started and eggs could be collected for the experiments. *Salix* shoots grown from 20-cm winter cuttings had between 17 and 35 leaves and were prepared by removing the top 2–4 newly emerged, incompletely unfolded leaves and the lowest old and withering leaves.

Analysis

The relationship between group size and egg predation risk was analysed separately for each predator type using a generalized linear mixed model (GLMM) framework with a binomial logit-link distribution. For this, the response variable was the number of eggs preyed upon from the 45 eggs within each group size category (i.e. number of 'successes' for a given number of 'trials'), with the individual plant ID as a random effect (Appendix C, Tab. C1). This was first implemented with group size treated as a categorical variable; thus providing separate estimates for each group size category. We used the predation risk per egg for the smallest group (clutch size = 5) as the baseline for calculations of attack-abatement for the other group sizes (Wrona & Dixon, 1991), where this level of predation risk would indicate no attack-abatement effect for larger groups (Fig. 1). From this we calculated the expected full attack-abatement effect on predation risk for each group size by dividing the

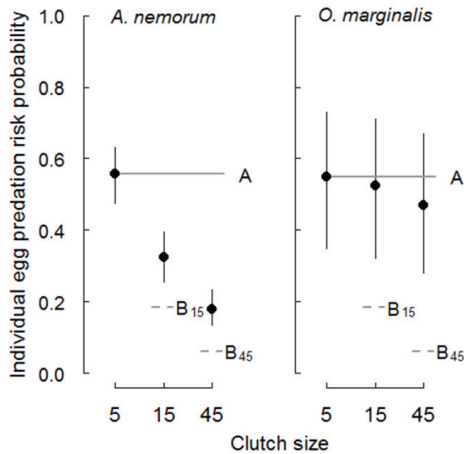


Figure 2: The attack-abatement effect for leaf beetle eggs relative to two different predator hunting strategies (*A. nemorum* = ‘eat and run’ versus *O. marginalis* = ‘find and stay’). Points show the median and 95% CIs of the posterior distributions of predation risk probability estimated from experimental observations for clutch sizes 5, 15 and 45 eggs (see Table C2). The advantage of grouping is calculated relative to the smallest group size (A) as the baseline, and the full potential advantage from attack-abatement for each group size (B15 & B45).

baseline risk by the relative increase in group size (i.e. 3 or 9 for group sizes of 15 and 45 respectively (Turner & Pitcher, 1986)). From these two reference points we could determine the proportion of the observed reduction in predation risk relative to the total possible reduction in predation risk (Fig. 1). For *A. nemorum*, because the proportion of prey taken per encounter is inversely related to group size (i.e. full dilution effect), if the observed attack-abatement is smaller than predicted this would indicate that increases in group size result in increased group encounters, with the proportionate increase being a direct measure of the change in the encounter effect relative to group size. For *O. marginalis*, because the proportion of prey taken is 1 (i.e. no dilution effect), we expect the observed predation risk to remain at a similar level regardless of group size. Because each group size represented a 3-fold increase in clutch size (5, 15 & 45), we also modelled the GLMM using group size coded as a continuous variable (0, 1, 2) to compare between-predator differences in intercepts (i.e. was there a baseline between-predator difference in predation risk for the smallest group size) and slopes (i.e. was there a between-predator difference in attack-abatement). We then extended these GLMM analyses to include plant sap quality/plant architecture, by including host plant species as a 2-category variable with interactions at the group level. From this we

wanted to examine how the quality of an additional food source or the plant architecture changed attack-abatement patterns depending on the degree of dilution in the predator hunting mode.

We estimated parameters and derived variables from the GLMMs using Bayesian hierarchical models (Appendix C, Tab. C3) implemented in JAGS (an MCMC Gibb’s sampler; (Plummer, 2003)) called from R (R Core Team 2015). We used a Bayesian framework primarily because we could generate posterior distributions for any derived variables of interest. This means that any variable calculated from the models (e.g. differences between predicted and observed values or proportions) has its own probability distribution from which the mean, standard deviation and 95% credible intervals can be calculated. This allows us to assign exact probabilities on whether things differ, rather than simply using point estimates that do not allow such interpretation. We used vague priors in all models, and sampled the MCMC chains 50 000 times once the chains had stabilized (for *A. nemorum* chains stabilized after 50 000 iterations; for *O. marginalis* chains took ~1 million iterations to stabilize). Convergence was checked by visually inspecting the chains and confirmed using the Gelman and Rubin diagnostic.

Results

Egg predation risk relative to group size and predator strategy

As predicted by attack-abatement theory there was a clear negative relationship between group size and egg predation risk when *A. nemorum* was the predator, while egg predation risk was largely independent of group size for *O. marginalis* (Fig. 2; Appendix C, Tab. C2). For *A. nemorum* there was strong evidence that larger groups were more likely to be encountered than smaller groups (45 > 15 > 5) because predation risk for larger groups was higher than predicted if encounter rate was independent of group size (Fig. 2); however, this higher encounter probability was too small to eliminate the benefits from the dilution effect. For intermediate-sized groups (15 eggs), attack-abatement was 63% of the expected effect if encounter rate was group-size-independent, and for the largest group (45 eggs) it was 76% of the expected full effect (Appendix C, Tab. C2).

Host plant identity and attack-abatement patterns

Attack-abatement patterns varied in relation to host plant species. Although the decline in predation risk relative to group size was largely similar on both *Salix* species, there was a clear difference in the absolute predation risk between *S. dasyclados* (higher quality

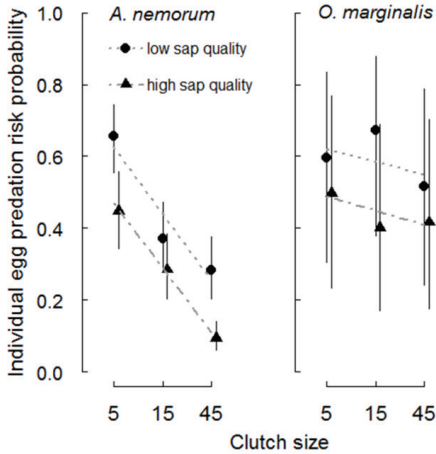


Figure 3: The attack-abatement effect for leaf beetle eggs relative to predator type (*A. nemorum* versus *O. marginalis*) and plant genotype (lower sap quality = *S. viminalis*; higher sap quality = *Salix dasyclados*) based on the derived variable estimates in Table S3. Points show the median and 95% CIs of the posterior distributions estimated from models where clutch size is a categorical variable; lines show the predicted median of models where clutch size was treated as a continuous variable (mind that for *A. nemorum* the slope of high sap quality is statistically steeper than for low sap quality).

sap for predators/simpler plant architecture = lower egg predation risk) and *S. viminalis* (lower quality sap/complex plant architecture = higher egg predation risk; Fig. 3). For *A. nemorum* there was strong evidence for differences in attack-abatement between *S. dasyclados* and *S. viminalis* based on a linear decline in predation risk relative to group size (Appendix C, Tab. C3). Here there was a 98% probability that *S. dasyclados* had a lower intercept than *S. viminalis* (difference between intercepts (logit mean \pm SD) = 0.62 ± 0.30 ; 95% CI = 0.03, 1.22), and a 99% probability that the attack-abatement effect was stronger on *S. dasyclados* (difference between slopes (logit) = 0.20 ± 0.08 , 95% CI = 0.05, 0.36). For *O. marginalis* the magnitude of the estimated mean difference between intercepts on the different *Salix* species was similar to *A. nemorum* (0.60 ± 0.87); however, there was much greater uncertainty in the estimates (only 75% probability that *S. dasyclados* < *S. viminalis* intercept; 95% CI = -1.1, 2.4; Fig. 3, Appendix C, Tab. C3). There was no difference between the slopes for the two *Salix* species with *O. marginalis* (mean \pm SD = 0.007 ± 0.09 ; 95% CI = -0.18, 0.17).

Discussion

Empirical studies about attack-abatement have been generally limited to: (1) single predator-prey systems, thus not examining how the relationship between group size and predation risk changes for a single prey species as the key components of attack-abatement are altered (i.e. encounter or dilution); (2) non-sessile prey (but see (Wrona & Dixon, 1991)), meaning that additional factors related to group living are likely to confound attack-abatement patterns and prevent disentangling the relative contribution of encounter and dilution in determining predation risk; and (3) environments where local resources or habitat quality outside of the predator-prey interaction are assumed to be neutral, meaning that studying how bottom-up processes interact with attack-abatement is still unexplored. By using a model prey system with two predators at the extreme ends of the dilution effect range, and an experimental design with the same total number of sessile prey ($n = 45$) in each group size category, we could tease apart the contribution of the encounter and dilution effect to attack-abatement in unusual detail. In addition, because we used two different host plant species to represent different impacts of habitat quality and complexity as the experimental arena we could show for the first time how top-down (hunting modes) and bottom-up (different suitability of plant genotypes to the omnivorous predators; different complex foraging areas) processes interacted to generate attack-abatement patterns.

By comparing the benefits of grouping when confronted with predators with different fixed hunting modes relating to prey consumption – one where the dilution effect is complete (constant prey number consumed regardless of group size) versus one where the dilution effect is absent (whole group is consumed) – we could show that attack-abatement predictions relating to variation in the dilution effect on a single prey species (Fig. 1, (Turner & Pitcher, 1986; Inman & Krebs, 1987)) were largely supported. The fact that prey benefit from the full dilution effect in the presence of *A. nemorum* also allowed us to quantify how the encounter effect contributes to lowering predation risk in larger groups. Because eggs are sessile prey we can be confident that attack-abatement is the mechanism lowering individual predation risk in groups, with attack-abatement acting in the absence of more complicated behavioural and group structure effects that usually overlay and strengthen/weaken the encounter and dilution mechanisms (e.g. vigilance or confusion (Roberts, 1996; Schradin, 2000)).

Because we could largely control for all other factors that contribute to the benefits of prey grouping behaviour, differences between observed and expected predation risks under the full encounter effect (i.e.

group encounter rates are the same regardless of group size) should accurately reflect the proportionate increase in group encounters that directly result from larger group size. Thus, a three-fold group size increase from 5 to 15 does not result in a reduction in predation risk of 0.37 ± 0.03 as predicted against *A. nemorum*, but rather 0.23 ± 0.02 (Fig. 2, Appendix C, Tab. C1). This indicates that approximately one third of the expected benefit of grouping is lost because of the increase in detectability or group encounter rate. Interestingly, a further three-fold increase in group size from 15 to 45 shows the same pattern; a reduction in predation risk with one third of the expected benefit lost because of an increase in encounter rate. Thus, the benefits of the encounter effect as group size increased remained relatively constant within the range of natural clutch sizes for the leaf beetle in these experiments.

The benefits of grouping do not occur when eggs are being hunted by *O. marginalis*. Here the dilution effect is zero because the probability of being consumed if encountered is 1; since attack-abatement is the product of the dilution and encounter effects, the benefits from grouping is zero (anything times zero is zero). Although recent studies have also looked at relationships between predator hunting behaviour and prey grouping (Cresswell & Quinn, 2010), their results are, as adult prey that can respond to the environment were investigated, influenced by vigilance and predator confusion effects that play a large role in the advantages of grouping in those species. From our study we can show that differences in hunting behaviour among predators also strongly translate into different survival probabilities of eggs. Thus, even with this passive sessile prey, the hunting behaviour determines if larger groups are advantageous or not.

Our experimental set up has removed some cues that are considered important for guiding the insect predators, resulting in attack-abatement patterns that might look different under natural conditions. Olfactometric cues such as long-range detection via herbivore-induced plant volatiles (Dicke & Baldwin, 2010) (reliability-detectability problem (Vet *et al.*, 1990)) or pheromones and faeces as foraging kairomones (Fernandez & Hilker, 2007) are important cues in prey detection. In aiming for a design with three distinct size classes with the same number of eggs by pinning clutches on otherwise healthy plants we excluded these cues; it has been shown that at least *A. nemorum* is attracted to herbivore-induced plant volatiles from plants attacked by *P. vulgatissima* (Lehrman *et al.*, 2013). This suggests the weakening of the encounter effect in larger groups observed in our study resulted from increased conspicuousness of egg clutches from visual detection (Hénaut *et al.*, 1999) and olfactometric detection of eggs themselves (Bin *et al.*, 1993), and not from plant-based volatiles.

Although this potentially complicates the interpretation of natural selection pressures on clutch size determination in these insects, one advantage of having removed these cues is that our results can be generalized to systems with predators mainly using vision (such as avian predators).

Unfortunately predator hunting mode was confounded by predator species, meaning that it could be argued our results come from a 'species' effect and not the hunting mode. We think this is unlikely for many reasons, not only that other studies find consistent evidence for predator hunting mode as an explanation for observed effects on prey survival and behaviour (Miller *et al.*, 2014). Besides mode, hunting domain is also important for prey survival. However, the different hunting domains of the two predators on the shoot should not confound hunting mode because: (i) the clutch size classes were distributed randomly along the shoot and survival was calculated per class, not clutch; (ii) both predators visited all positions on the shoot (not shown); (iii) plant sap quality does not change along the shoot (Siebrecht *et al.*, 2003) and any differences in duration of feeding on plant sources would only alter the total egg consumption but not the predation risk probability in the different group size categories.

Another objective of this study was to investigate if there were bottom up effects modulating the outcome of attack-abatement. Certainly specific biotic (e.g. grass cover (Schaller, 1968)) and abiotic (e.g. light level (Metcalfe & Ure, 1995)) conditions during predator attack and the timing in relation to group formation completion (Morrell *et al.*, 2011) affect benefits of group living. However, these effects act on the more complex behaviours that overlay the benefits due to attack-abatement. In contrast, here we could exclude most of these complex behaviours and evaluated how either plant sap quality or plant architecture modulates attack-abatement. We believe that plant architecture plays a very minor role in these differences because: (i) both predators move very fast and had enough time during the study to visit every leaf on the shoot several times (e.g.: eggs were consumed on every vertical position), (ii) the leaf area on both genotypes was similar, and (iii) neither of the predators show behaviours that favour particular areas on the leaf (e.g. like exclusively following leaf edges). More importantly, one would expect that the area searched and the encounter and re-encounter of eggs would be greater on simple plant structures (Gingras *et al.*, 2008), which should increase predation pressure. In contrast, we observed lower predation on *S. dasyclados* with simpler structure, but higher sap quality meaning if there is an effect of architecture it is largely overshadowed by plant sap quality acting as bottom up effect on the omnivorous predators.

Although it is widely accepted that bottom up effects are important direct drivers for prey aggregations (Jensen & Larsson, 2002), here we considered for the first time how the benefits of prey aggregations are indirectly affected by bottom up effects – the modulation of predator behaviour due to switching to feeding on the same trophic level as the prey. Thus, the probability of group encounter was lower when the quality of the alternative food source was higher. This suggests that predators are less motivated to seek out prey groups if they can more easily satisfy their nutritional needs via other means. It is likely that our results could imply a more general phenomenon with important implications in that the relative benefits of prey grouping behaviour for any species could be expected to show diurnal or seasonal changes as other aspects of resource/habitat quality change for the focal predator.

We also found evidence that the slope of the regression describing the decline in predation risk with group size was steeper when alternative resources for the predator *A. nemorum* were of higher quality. Thus additional benefits of grouping may occur when predators are less motivated to hunt, with attack-abatement being less effective during periods when predators must satisfy their needs by hunting the focal prey.

Our results further indicate that leaf beetles should, in general, increase clutch size to increase egg survival; however observations of egg laying in this leaf beetle does not reveal such a clear pattern, as clutch size varies from 1-50 eggs and is not affected by predator presence (J. Stephan, unpublished data). There could be a number of explanations for this variation. First, is that by examining the relationship between grouping and predation risk in relation to different natural predators of the leaf beetle it is obvious that in a system with both *A. nemorum* and *O. marginalis* the benefits of attack-abatement would be weakened. For this leaf beetle it has been shown that it faces several predators in plantations as well as in natural willow stands and the lack of positive effects of larger clutches may be attributed to additive predator effects (Björkman *et al.*, 2003; Stephan *et al.*, 2016). We now can explain, by using predators with large difference in hunting mode, why it is difficult to detect benefits of larger clutches. This highlights an important point that is often not discussed: most studies to date have focused on a specific predator-prey interaction (e.g. Ioannou *et al.*, 2011) and have ignored the likely scenario that grouping strategies are not simply a result of the interaction under study but a compromise between several predator-prey interactions that may have different attack-abatement patterns (but see (Morrell *et al.*, 2011) on how different timings of predator attack, which correlates with hunting mode,

can affect group formation strategies). Second, there are complex interactions between food preferences for the ovipositing leaf beetles (that prefer *S. viminalis* (Stenberg *et al.*, 2011)) and those of their predators that result in trade-offs between food quality for the prey and predation risk avoidance. There are also implications for tritrophic – predator-prey-plant – interactions in that if the plant can satisfy the predator's nutritional needs with its sap, the predator is less likely to act as the plant's 'bodyguard' and hunt leaf beetles. Thus we should perhaps not be surprised that the leaf beetle does not follow the simple rule of 'lay big clutches' when there are so many interactions and selective pressures still unaccounted for.

Not only is this system an excellent model for studying attack-abatement, but also it has implications for optimal clutch size theory. Optimal clutch size in birds and other species is largely based on parental effort (although predation plays a key role; e.g. see (Eggers *et al.*, 2006)) while clutch size in invertebrates is more a description of how many eggs to lay in a particular place (Godfray *et al.*, 1991). Based on our findings ovipositing insects also need to consider benefits from attack-abatement either by laying larger clutches or by clustering eggs with those of other females. Clustering behaviours exist in form of superoviposition (Godfray *et al.*, 1991), double oviposition (Nagelkerke *et al.*, 1996), or communal egg laying in reptiles and amphibians (Doody *et al.*, 2009) and will also have to consider the selfish herd effect. In addition, leaf beetles use spatial memory to modify the between-clutch distance on a plant to lower intraspecific competition between sib/non-sib larvae and may be more sensitive to indirectly perceived conspecific density than directly perceived number of eggs on a plant (Stephan *et al.*, 2015). Predation risk avoidance may therefore be traded off against intraspecific competition avoidance. In insect ecology aggregations (of eggs and larvae) are explained by e.g. thermoregulation (Klok & Chown, 1999), overcoming plant defences (Clark & Faeth, 1997), or overwhelming predators (Ronnäs *et al.*, 2010). The application of attack-abatement to this field is still underdeveloped and would greatly benefit by including long-known attack-abatement effects on prey grouping. Although the classic studies on attack-abatement were over 20 years ago (Calvert *et al.*, 1979; Foster & Treherne, 1981; Turner & Pitcher, 1986; Inman & Krebs, 1987), there is still many unexplored aspects of how group formation relates to predation risk in terms of the key components of group encounter rate and numerical dilution. Using a simple system lacking many behavioural complications we have illustrated how elementary encounter effect and numerical dilution effect are, and propose future research to regard other behaviours and physiological constraints as

modulators that strengthen or weaken these effects. Modulation of attack-abatement by bottom-up effects such as plant based food resources is yet to be incorporated into general theory, despite omnivorous predators being more common than strict carnivores (Rosenheim & Corbett, 2003) and omnivory being important for shaping food webs (Holt & Polis, 1997), influencing ecosystem functions (Zhang *et al.*, 2004) and in biological control (Wäckers *et al.*, 2005). Thus, ongoing refinement of attack-abatement theory by focusing on bottom-up versus top-down processes, often in a tritrophic interaction perspective, could have significant impacts on many important contemporary fields of study.

Acknowledgement

We thank Stig Larsson and Barbara Ekbohm for comments on a very earlier version of the manuscript. This study was funded by the Swedish Energy Agency (Energimyndigheten) and the Swedish Research Agency (Formas).

Literature cited

- Abrams, P. A. & Ginzburg, L. R. (2000). The nature of predation: Prey dependent, ratio dependent or neither? *Trends in Ecology and Evolution*, 15(8), pp 337–341.
- Bednekoff, P. a. & Lima, S. L. (1998). Re-examining safety in numbers: interactions between risk dilution and collective detection depend upon predator targeting behaviour. *Proceedings of the Royal Society B: Biological Sciences*, 265(July), pp 2021–2026.
- Bin, F., Vinson, S. B., Strand, M. R., Colazza, S. & W.A.Jr, J. (1993). Source of an egg kairomone for *Trissolcus basalus*, a parasitoid of *Nezara viridula*. *Physiological Entomology*, 18(1), pp 7–15.
- Björkman, C., Dalin, P. & Eklund, K. (2003). Generalist natural enemies of a willow leaf beetle (*Phratora vulgatissima*): abundance and feeding habits. *Journal of Insect Behavior*, 16(6), pp 747–764.
- Björkman, C., Johansson, H. & Snäll, T. (2011). Spatial distribution of interacting insect predators: Possible roles of intraguild predation and the surrounding habitat. *Basic and Applied Ecology*, 12(6), pp 516–522.
- Calvert, W. H., Hedrick, L. E. & Brower, L. P. (1979). Mortality of the Monarch Butterfly (*Danaus plexippus* L.): Avian Predation at Five Overwintering Sites in Mexico. *Science*, 204(4395), pp 847–851.
- Clark, B. & Faeth, S. (1997). The consequences of larval aggregation in the butterfly *Chlosyne lacinia*. *Ecological Entomology*, 22(4), pp 408–415.
- Cocroft, R. B. (1999). Parent-Offspring Communication in Response to Predators in a Subsocial Treehopper (Hemiptera: Membracidae: *Umberonia crasicornis*). *Ethology*, 105, pp 553–568.
- Creel, S. & Winnie, J. a. (2005). Responses of elk herd size to fine-scale spatial and temporal variation in the risk of predation by wolves. *Animal Behaviour*, 69(5), pp 1181–1189.
- Cresswell, W. & Quinn, J. L. (2010). Attack frequency, attack success and choice of prey group size for two predators with contrasting hunting strategies. *Animal Behaviour*, 80(4), pp 643–648 Elsevier Ltd.
- Dalin, P., Kindvall, O. & Björkman, C. (2009). Reduced population control of an insect pest in managed willow monocultures. *PloS one*, 4(5), p e5487.
- Davies, N. B., Krebs, J. R. & West, S. A. (2012). *An Introduction to Behavioural Ecology*. John Wiley & Sons. ISBN 0632035463.
- Dicke, M. & Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the "cry for help". *Trends in Plant Science*, 15(3), pp 167–75 Elsevier Ltd.
- Doody, J. S., Freedberg, S. & Keogh, J. S. (2009). Communal egg-laying in reptiles and amphibians: evolutionary patterns and hypotheses. *The Quarterly Review of Biology*, 84(3), pp 229–252.
- Ebensperger, L. a. (2001). A review of the evolutionary causes of rodent group-living. *Acta Theriologica*, 46(2), pp 115–144.
- Eggers, S., Griesser, M., Nystrand, M. & Ekman, J. (2006). Predation risk induces changes in nest-site selection and clutch size in the Siberian jay. *Proceedings of the Royal Society of London B: Biological Sciences*, 273(1587), pp 701–706.
- Fernandez, P. & Hilker, M. (2007). Host plant location by Chrysomelidae. *Basic and Applied Ecology*, 8(2), pp 97–116.
- Foster, W. A. & Treherne, J. E. (1981). Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. *Nature*.
- Gingras, D., Dutilleul, P. & Boivin, G. (2008). Effect of plant structure on searching strategy and searching efficiency of *Trichogramma turkestanica*. *Journal of Insect Science*, 8(28), pp 1–9.
- Godfray, H. C. J. (1986). Clutch size in a leaf-mining fly (*Pegomya nigrotarsis*:

- Anthomyiidae). *Ecological Entomology*, 11, pp 75–81.
- Godfray, H. C. J., Partridge, L. & Harvey, P. H. (1991). Clutch Size. *Annual Review of Ecology and Systematics*, 22(1), pp 409–429.
- Grevstad, F. S. & Klepetka, B. W. (1992). The influence of plant architecture on the foraging efficiencies of a suite of ladybird beetles feeding on aphids. *Oecologia*, 92(3), pp 399–404.
- Hamilton, W. D. (1971). Geometry for the selfish herd. *Journal of Theoretical Biology*, 31(2), pp 295–311.
- Hénaut, Y., Alauzet, C., Dargagnon, D. & Lambin, M. (1999). Visual learning in larval Orius majusculus a polyphagous predator. *Entomologia Experimentalis et Applicata*, 90(1), pp 103–107.
- Hendrichs, J., Katsoyannos, B. I., Papaj, D. R. & Prokopy, R. J. (1991). Sex-Differences in Movement Between Natural Feeding and Mating Sites and Tradeoffs Between Food-Consumption, Mating Success and Predator Evasion in Mediterranean Fruit-Flies (Diptera, Tephritidae). *Oecologia*, 86(2), pp 223–231.
- Holt, R. D. & Polis, G. a (1997). A theoretical framework for intraguild predation. *The American Naturalist*, 149, pp 745–764.
- Inman, A. J. & Krebs, J. (1987). Predation and group living. *Trends in Ecology & Evolution*, 2(2), pp 31–32.
- Ioannou, C. C., Bartumeus, F., Krause, J. & Ruxton, G. D. (2011). Unified effects of aggregation reveal larger prey groups take longer to find. *Proceedings of the Royal Society of London B: Biological Sciences*, 278(1720), pp 2985–2990.
- Jensen, K. H. & Larsson, P. (2002). Predator evasion in Daphnia: The adaptive value of aggregation associated with attack abatement. *Oecologia*, 132(3), pp 461–467.
- Kagata, H. & Ohgushi, T. (2002). Clutch size adjustment of a leaf-mining moth (Lyonetiidae: Lepidoptera) in response to resource availability. *Ecology and Population Biology*, 95(2), pp 213–217.
- Klok, C. J. & Chown, S. L. (1999). Assessing the benefits of aggregation: thermal biology and water relations of anomalous Emperor Moth caterpillars. *Functional Ecology*, 13(3), pp 417–427.
- Krause, J. & Ruxton, G. (2002). *Living in groups*. Oxford: Oxford University Press.
- Lehman, H. (1932). Wanzen (Hemiptera-Heteroptera) als Obstbaumschädlinge. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, 42, pp 440–451.
- Lehrman, A., Boddum, T., Stenberg, J. A., Orians, C. M. & Björkman, C. (2013). Constitutive and herbivore-induced systemic volatiles differentially attract an omnivorous biocontrol agent to contrasting Salix clones. *AoB plants*, 5, p plt005.
- Lehrman, A., Torp, M., Stenberg, J. A., Julkunen-Tiitto, R. & Björkman, C. (2012). Estimating direct resistance in willows against a major insect pest, Phratora vulgatissima, by comparing life history traits. *Entomologia Experimentalis et Applicata*, 144(1), pp 93–100.
- Maschwitz, U. W. (1966). Alarm substances and alarm behavior in social insects. *Vitamins and hormones*, 24, pp 267–290.
- Metcalfe, N. B. & Ure, S. E. (1995). Diurnal Variation in Flight performance and hence potential predation risk in small birds. *Proceedings of the Royal Society B Biological Sciences*, 261, pp 395–400.
- Miller, J. R. B., Ament, J. M. & Schmitz, O. J. (2014). Fear on the move: predator hunting mode predicts variation in prey mortality and plasticity in prey spatial response. *Journal of Animal Ecology*, 83(1), pp 214–222.
- Morrell, L. J., Ruxton, G. D. & James, R. (2011). The temporal selfish herd: predation risk while aggregations form. *Proceedings. Biological sciences / The Royal Society*, 278(1705), pp 605–612.
- Nagelkerke, C. J., Baalen, M. & Sabelis, M. W. (1996). When should a female avoid adding eggs to the clutch of another female? A simultaneous oviposition and sex allocation game. *Evolutionary Ecology*, 10(5), pp 475–497.
- Plummer, M. (2003). JAGS: a program for analysis of Bayesian graphical models using Gibbs sampling. *Proceedings of the 3rd International Workshop on Distributed Statistical Computing (DSC 2003), March 20–22, Vienna, Austria. ISSN 1609-395X.*,
- Pollard, K. a. & Blumstein, D. T. (2008). Time allocation and the evolution of group size. *Animal Behaviour*, 76(5), pp 1683–1699.
- Roberts, G. (1996). Why individual vigilance declines as group size increases. *Animal Behaviour*, 51(June 1995), pp 1077–1086.
- Ronnäs, C., Larsson, S., Pitacco, A. & Battisti, A. (2010). Effects of colony size on larval performance in a processionary moth. *Ecological Entomology*, 35, pp 436–445.
- Rosenheim, J. A. & Corbett, A. (2003). Omnivory and the indeterminacy of predator function:

- Can a knowledge of foraging behavior help? *Ecology*, 84(10), pp 2538–2548.
- Schaller, G. B. (1968). Hunting behaviour of the cheetah in the Serengeti National Park, Tanzania. *African Journal of Ecology*, 6(1), pp 95–100.
- Schmitz, O. (2007). Predator diversity and trophic interactions. *Ecology*, 88(10), pp 2415–2426.
- Schradin, C. (2000). Confusion effect in a reptilian and a primate predator. *Ethology*, 106(8), pp 691–700.
- Siebrecht, S., Herdel, K., Schurr, U. & Tischner, R. (2003). Nutrient translocation in the xylem of poplar? diurnal variations and spatial distribution along the shoot axis. *Planta*, 217(5), pp 783–793.
- Stenberg, J. A., Lehrman, A. & Björkman, C. (2010). Uncoupling direct and indirect plant defences: Novel opportunities for improving crop security in willow plantations. *Agriculture, Ecosystems and Environment*, 139(4), pp 528–533.
- Stenberg, J. a., Lehrman, A. & Björkman, C. (2011). Host-plant genotype mediates supply and demand of animal food in an omnivorous insect. *Ecological Entomology*, 36(4), pp 442–449.
- Stephan, J. G., Albertsson, J., Wang, L. & Porcel, M. (2016). Weeds within willow short-rotation coppices alter the arthropod community and improve biological control of the blue willow beetle. *BioControl*, 61(1), pp 103–114.
- Stephan, J. G., Stenberg, J. A. & Björkman, C. (2015). How far away is the next basket of eggs? Spatial memory and perceived cues shape aggregation patterns in a leaf beetle. *Ecology*, 96(4), pp 908–914.
- Subinprasert, S. & Svensson, B. W. (1988). Effects of predation on clutch size and egg dispersion in the codling moth *Laspeyresia pomonella*. *Ecological Entomology*, 13, pp 87–94.
- Turner, G. F. & Pitcher, T. J. (1986). Attack Abatement: A Model for Group Protection by Combined Avoidance and Dilution. *The American Naturalist*, 128(2), p 228 JSTOR.
- Uetz, G. W. & Hieber, C. S. (1994). Group size and predation risk in colonial web-building spiders: analysis of attack abatement mechanisms. *Behavioral Ecology*, 5(3), pp 326–333.
- Vasseur, D. a & Fox, J. W. (2011). Adaptive dynamics of competition for nutritionally complementary resources: character convergence, displacement, and parallelism. *The American Naturalist*, 178(4), pp 501–514.
- Vet, L. E. M., Dicke, M. & Wäckers, F. L. (1990). How To Hunt for Hiding Hosts: the Reliability-Detectability Problem in Foraging Parasitoids. *Netherlands Journal of Zoology*, 41(2), pp 202–213 Brill.
- Wäckers, F. L., Rijn, P. C. J. van & Bruin, J. (2005). *Plant-Provided Food for Carnivorous Insects: a Protective Mutualism and Its Applications*. Cambridge University Press. ISBN 0521819415.
- Weih, D. & Webb, P. (1984). Optimal avoidance and evasion tactics in predator-prey interactions. *Journal of Theoretical Biology*, 106(2), pp 189–206.
- Wiedenmann, R. & O'Neil, R. (1992). Searching strategy of the predator *Podisus maculiventris* (Say)(Heteroptera: Pentatomidae). *Environmental Entomology*, 21(1), pp 1–9.
- Wrona, F. J. & Dixon, R. W. J. (1991). Group Size and Predation Risk: A Field Analysis of Encounter and Dilution Effects. *The American Naturalist*, 137(2), p 186.
- Zhang, Y., Richardson, J. S. & Negishi, J. N. (2004). Detritus processing, ecosystem engineering and benthic diversity: A test of predator-omnivore interference. *Journal of Animal Ecology*, 73(4), pp 756–766.

Appendix

Definition of dilution and encounter effect

Attack-abatement consists of two effects, each having a range of possible conditions: the complete encounter effect (or avoidance effect (Turner and Pitcher 1986)) describing that the probability of an predator encountering an individual is lower in a group of prey than for an equal number of solitary prey individuals if the predator exhibits random search behavior (Brock and Riffenburgh 1960, Vine 1971, Treisman 1975, Turner and Pitcher 1986, Uetz and Hieber 1994). This situations only occurs if the probability of detection is independent of group size (Treisman 1975) meaning larger groups are not more conspicuous to a predator. This can partly be due to the visual apparency effect (Vine 1971) which describes that detection probability levels off above a critical group size because the predator is only seeing a two-dimensional image of a three-dimensional group. The encounter effect can also only be partial because grouping can lead to increased predation risk because larger groups can also be more attractive and/or easier to find by predators (Vine 1973, Bertram 1978). This aggregative response effect (Hassell and May 1974) or increased conspicuousness has been observed in several different orders of

organism (Rypstra 1979, Hixon 1997, Creel and Creel 2002).

If the group is encountered, the dilution effect (a.k.a. numerical 'dilution' (Turner and Pitcher 1986, Wrona and Dixon 1991); contrary to more unspecific use of dilution to describe increased safety with increasing number of conspecifics) predicts higher chances of survival for individuals in a larger group than in a smaller group (Foster and Treherne 1981). In a situation where the predator is able to consume all discovered prey the dilution effect is zero while complete dilution occurs if the predator consumes a constant, group size independent number of individuals which may originate in predator satiation (Bertram 1978) or prey evasion (Treisman 1975). Partial dilution occurs because the proportion of the group taken is less as group size increases with the predation risk increases non-linearly with group size. The dilution effect and its positive effect for larger groups can be amplified if the prey is defending itself (Tostowaryk 1972), the aggregated prey intimidates (Rahel and Stein 1988) or confuses the predator (Milinski 1977). Assuming, in contrast, that the predation risk is not divided equally among the group members because individuals might try to minimize their domain of danger by e.g. seeking a central position within the group led to the formulation of the selfish-herd model (Hamilton 1971).

Going through the relevant literature we noticed that the terms dilution and encounter are used differently. Larger groups can generally dilute the risk of predation. However, care should be taken to separate these general statement from the numerical dilution effect. Also, the encounter effect is by definition base on a finite number of preys within a predator search area forming groups or not (Turner and Pitcher 1986) and claiming otherwise and making a strong distinguishing between conspicuousness and encounter (Ioannou et al. 2011) can be confusing, at least. Similar to the definition by Turner and Pitcher that described a complete encounter effect (e.g. a group of 200 gazelles are as likely to be encountered than one gazelle (Inman and Krebs 1987)) and a zero encounter effect (less frequent encounter of larger groups is completely swapped by higher conspicuousness) we suggest using dilution effect and encounter effect as the basic mechanisms of attack-abatement with all other behavioral mechanisms and physiological limitations modulating these effects. Therefore other effects like prey vigilance followed by predator evasion (weakening of encounter effect) or group defenses (weakening of dilution effect) could probably be incorporated leading to a more thorough understanding of grouping in general.

Literature cited

- Bertram, B. C. (1978). Living in groups: predators and prey. In: Krebs, J. R. & Davies, N. B. (Eds) *Behavioural ecology: an evolutionary approach*. pp 64–96. Blackwell, Oxford.
- Brock, V. & Riffenburgh, R. (1960). Fish schooling: a possible factor in reducing predation. *ICES Journal of Marine Science*, 25(3), pp 307–317.
- Creel, S. & Creel, N. M. (2002). *The African Wild Dog: Behavior, Ecology, and Conservation*. ISBN ISBN13: 978-0-691-01654-2.
- Foster, W. A. & Treherne, J. E. (1981). Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. *Nature*.
- Hamilton, W. D. (1971). Geometry for the selfish herd. *Journal of Theoretical Biology*, 31(2), pp 295–311.
- Hassell, M. P. & May, R. M. (1974). Aggregation of Predators and Insect Parasites and its Effect on Stability. *Journal of Animal Ecology*.
- Hixon, M. A. (1997). Synergistic Predation, Density Dependence, and Population Regulation in Marine Fish. *Science*.
- Inman, A. J. & Krebs, J. (1987). Predation and group living. *Trends in Ecology & Evolution*, 2(2), pp 31–32.
- Ioannou, C. C., Bartumeus, F., Krause, J. & Ruxton, G. D. (2011). Unified effects of aggregation reveal larger prey groups take longer to find. *Proceedings of the Royal Society of London B: Biological Sciences*, 278(1720), pp 2985–2990.
- Milinski, M. (1977). Experiments on the Selection by Predators against spatial Oddity of their Prey. *Zeitschrift für Tierpsychologie*, 43(3), pp 311–325.
- Rahel, F. & Stein, R. (1988). Complex predator-prey interactions and predator intimidation among crayfish, piscivorous fish, and small benthic fish. *Oecologia*, 75, pp 94–98.
- Rypstra, A. L. (1979). Foraging flocks of spiders. *Behavioral Ecology and Sociobiology*.
- Tostowaryk, W. (1972). The effect of prey, defense and the functional response of *Podisus modestus* (Hemiptera: Pentatomidae) to densities of the sawflies *Neodiprion swainei* and *N. pratti banksianae* (Hymenoptera: Neodiprionidae). *The Canadian Entomologist*, 104, pp 61–69.
- Treisman, M. (1975). Predation and the evolution of gregariousness. I. Models for concealment and evasion. *Animal Behaviour*, 23, pp 779–800.
- Turner, G. F. & Pitcher, T. J. (1986). *Attack Abatement: A Model for Group Protection by Combined Avoidance and Dilution*. *The American Naturalist*, 128(2), p 228 JSTOR.
- Uetz, G. W. & Hieber, C. S. (1994). Group size and predation risk in colonial web-building spiders:

- analysis of attack abatement mechanisms. Behavioral Ecology, 5(3), pp 326–333.
- Vine, I. (1971). Risk of visual detection and pursuit by a predator and the selective advantage of flocking behaviour. Journal of Theoretical Biology, 30(2), pp 405–422.
- Vine, I. (1973). Detection of prey flocks by predators. Journal of Theoretical Biology, 40, pp 207–210.
- Wrona, F. J. & Dixon, R. W. J. (1991). Group Size and Predation Risk: A Field Analysis of Encounter and Dilution Effects. The American Naturalist, 137(2), p 186.

Parameter estimates, variable estimates for Fig 2, variable estimates for Fig 3

Table 1: Parameter estimates (\pm SE) and their 95% credible intervals for models describing the predation risk per egg for leaf beetles under the threat of predation from either *A. nemorum* or *O. marginalis*. Estimates are from binomial (logit link) generalized linear mixed models where C_5 is the reference category (intercept) of group size = 5, with C_{15} and C_{45} the deviations from this for group sizes 15 and 45 respectively. Sigma is the standard deviation of the plant ID random effect on the intercept. Estimates and 95% CIs are generated from a Bayesian Gibb's sampler based on 50 000 draws.

Parameter	<i>A. nemorum</i>		<i>O. marginalis</i>	
	Estimate	95% CIs	Estimate	95% CIs
C_5	0.23 \pm 0.17	-0.10, 0.55	0.21 \pm 0.42	-0.62, 1.04
C_{15}	-0.96 \pm 0.07	-1.11, -0.82	-0.10 \pm 0.09	-0.28, 0.07
C_{45}	-1.74 \pm 0.08	-1.89, -1.58	-0.32 \pm 0.09	-0.50, -0.15
Sigma	1.02 \pm 0.12	0.81, 1.30	2.42 \pm 0.35	1.83, 3.23

Table 2: Derived variable estimates (mean \pm SD) and their 95% credible intervals from models describing the predation risk per egg for leaf beetles under the threat of predation from either *A. nemorum* or *O. marginalis*. Estimates are generated from the posterior distributions of the variables in models from Table S3, with the 'reference' and 'observed' variables representing the points and lines shown in Fig. 2. The absolute and relative differences in predation risk between the maximum expected advantages from grouping (B) and the observed predation risk (C) are shown relative to the smallest group size (A) for group sizes of 15 (B15 & C15 respectively) and 45 (B45 & C45 respectively).

Derived variable	<i>A. nemorum</i>		<i>O. marginalis</i>	
	Estimate	95% CIs	Estimate	95% CIs
Reference				
A (C_5 observed)	0.55 \pm 0.04	0.47, 0.64	0.55 \pm 0.09	0.35, 0.73
B ₁₅	0.18 \pm 0.02	0.15, 0.21	0.18 \pm 0.03	0.12, 0.25
B ₄₅	0.06 \pm 0.01	0.05, 0.07	0.06 \pm 0.01	0.04, 0.08
Observed				
C ₁₅	0.32 \pm 0.04	0.26, 0.40	0.52 \pm 0.09	0.33, 0.72
C ₄₅	0.18 \pm 0.03	0.13, 0.23	0.47 \pm 0.09	0.28, 0.67
Absolute Difference				
A–B ₁₅	0.37 \pm 0.03	0.31, 0.42	0.37 \pm 0.07	0.23, 0.49
A–B ₄₅	0.49 \pm 0.03	0.42, 0.56	0.49 \pm 0.09	0.31, 0.66
A–C ₁₅	0.23 \pm 0.02	0.20, 0.27	0.02 \pm 0.02	-0.02, 0.06
A–C ₄₅	0.37 \pm 0.02	0.33, 0.42	0.07 \pm 0.02	0.03, 0.12
Relative Difference				
A–C ₁₅ / A–B ₁₅	0.63 \pm 0.05	0.53, 0.72	0.06 \pm 0.06	-0.05, 0.19
A–C ₄₅ / A–B ₄₅	0.76 \pm 0.03	0.70, 0.81	0.16 \pm 0.05	0.07, 0.27

Table 3: Derived variable estimates (mean \pm SD of the posterior distribution) and their 95% credible intervals describing the predation risk per egg for leaf beetles under the threat of predation from either *A. nemorum* or *O. marginalis* on host plant genotypes that varied in sap quality for the omnivorous predator (higher quality sap = *Salix dasyclados*; lower quality sap = *S. viminalis*). Estimates are from binomial (logit link) generalized linear mixed models with plant genotype*group size interactions, where the group size effect was estimated either as a categorical variable (group sizes 5, 15 and 45 correspond to C5, C15 and C45 respectively) or as a continuous variable (group sizes 5, 15 and 45 correspond to 0, 1 and 2 respectively) with an intercept and slope. Posterior distributions were generated from a Bayesian Gibb's sampler based on 50 000 draws.

Derived variable	<i>A. nemorum</i>		<i>O. marginalis</i>	
	Estimate	95% CIs	Estimate	95% CIs
<u>Higher quality sap</u>				
C ₅	0.45 \pm 0.05	0.34, 0.56	0.49 \pm 0.14	0.23, 0.76
C ₁₅	0.29 \pm 0.05	0.20, 0.38	0.41 \pm 0.13	0.17, 0.69
C ₄₅	0.09 \pm 0.02	0.06, 0.14	0.42 \pm 0.13	0.17, 0.70
<hr/>				
Intercept	-0.12 \pm 0.21	-0.54, 0.30	-0.06 \pm 0.60	-1.26, 1.12
Slope	-0.99 \pm 0.06	-1.11, -0.88	-0.16 \pm 0.06	-0.28, -0.05
<hr/>				
<u>Lower quality sap</u>				
C ₅	0.65 \pm 0.05	0.55, 0.74	0.59 \pm 0.14	0.31, 0.83
C ₁₅	0.37 \pm 0.05	0.27, 0.47	0.66 \pm 0.14	0.38, 0.87
C ₄₅	0.29 \pm 0.04	0.20, 0.38	0.52 \pm 0.14	0.24, 0.78
<hr/>				
Intercept	0.50 \pm 0.21	0.08, 0.93	0.53 \pm 0.62	-0.66, 1.78
Slope	-0.79 \pm 0.05	-0.89, -0.69	-0.16 \pm 0.07	-0.29, -0.02

Consumptive and nonconsumptive effect ratio depends on local resource quality – a story about host plants, leaf beetles, and omnivorous predators

Jörg G. Stephan^{1*}, Johan A. Stenberg², and Christer Björkman¹

¹ Department of Ecology, Unit of Forest Entomology, Swedish University of Agricultural Sciences, PO Box 7044, SE-75007 Uppsala, Sweden

² Department of Plant Protection Biology, Unit of Integrated Plant Protection, Swedish University of Agricultural Sciences, PO Box 102, SE-23053 Alnarp, Sweden

*Corresponding author e-mail: jorg.stephan@slu.se

Abstract

Predators do not only consume prey but also exert nonconsumptive effects in form of scaring and disturbing processes like feeding or reproduction. Within insect ecology recently attempts have been made to link nonconsumptive effects of predators to the variation in local resource quality in form of plant genotypes that differ in suitability to the herbivore but also the plant sap feeding omnivorous predator. However, as with nonconsumptive effects in general, the direct link to the prey fitness is rarely made and results come mostly from one predator with its particular foraging behavior. Within the tritrophic system of a leaf beetle, two omnivorous bugs with different hunting modes, and four willow genotypes we therefore investigated how many eggs the beetle lays in predator presence and absence on different host plants. We calculated the contribution of consumptive (eggs predated) and nonconsumptive (lowered oviposition rate of the beetle) effects (c:nc-ratio) on beetle fitness and found that natural variation in host plant quality led to nonconsumptive effects ranging from one third to two and a half times as strong as predation. We also investigated the beetles mean and distribution of clutch sizes and found that females lower future intraspecific competition among larvae by laying generally smaller, but also more smaller than larger clutches on unsuitable plant genotypes. While larger clutches increase individual egg survival in presence of the predator exhibiting the hunting mode of not consuming all encountered eggs, the females responded unexpected similar to all predator treatments in not lowering mean, but median clutch size. We emphasize to investigate different predators as they can respond differently, and allow herbivore and predator behavior in assessing biocontrol strategies. Looking at key life history traits for reproduction and their modulation by bottom-up and top-down effects will help to understand how and why species aggregate.

Keywords: tritrophic interaction, oviposition rate, antipredator behavior, non-lethal predator effects, trait-mediated effects, predator–prey interactions, omnivore, clutch size, non-consumptive effects

Introduction

Top-down effects of predators consist of two components: a direct consumptive effect and a nonconsumptive effect associated with changes in prey behavior. The nonconsumptive effect can have a far-reaching impact on trophic cascades (Beckerman *et al.*, 1997; Trussell *et al.*, 2003), ecosystem functions (Schmitz *et al.*, 2008; Matassa & Trussell, 2011), and often equals or exceeds the effects of direct consumption (Schmitz *et al.*, 2004; Preisser *et al.*, 2005). Nonconsumptive effects can increase prey vulnerability to other mortality factors (McCauley & Rowe, 2011) or generate physiological stress, resulting in energetic costs cascading a negative impact on prey reproduction (Nelson *et al.*, 2004; Nelson, 2007; Creel *et al.*, 2009). A lower reproduction should represent the strongest nonconsumptive effect as it lowers prey fitness and is exerted by for example mating

interruption (Travers & Sih, 1991), higher conspicuousness of males attracting females (Uzendoski *et al.*, 1993), or changes in prey behavior that result in lower weight gain or poorer provision of progeny (Harfenist & Ydenberg, 1995). Because all these nonconsumptive effects change behavioral traits of the prey, they are termed trait-mediated interactions in contrast to direct consumption as a density mediated interaction (Preisser *et al.*, 2005).

Here we will focus on the egg laying behavior of an herbivorous leaf beetle and the traits clutch size and oviposition rate and if they are affected by predator presence. We will also investigate if these traits are altered by bottom-up effects (represented by host plants of different quality) and how the bottom-up effects on the herbivore and on the omnivorous predator interacts with the nonconsumptive effects.

Bottom-up effects on herbivores depend on plant quality (Denno *et al.*, 2002), including plant resistance, involving different combinations of defense traits

(Agrawal, 2007; Schaller, 2008; Karban, 2011). Variation in plant quality to a herbivore exist not only at the level of species, but also among plant genotypes (Kaplan & Thaler, 2010; Stenberg *et al.*, 2011a) and affect herbivore performance (Kaplan & Thaler, 2010), fitness (Lehrman *et al.*, 2012) and ultimately community composition (Schmitz *et al.*, 2008; Wimp *et al.*, 2010). The effect of the plant genotype on trophic levels above the herbivores should be important as predation depends on structural features like trichomes (Mulatu *et al.*, 2006) and chemical features of plants like volatiles that differ among plant genotypes (Degen *et al.*, 2004). Additionally, many insect predators are omnivores and differing plant sap quality of the genotype can alter the means to satisfy their nutritional needs leading to higher or lower consumption of animal prey (Lundgren *et al.*, 2009; Stenberg *et al.*, 2011b). The impact of plant genotype on higher trophic levels has been addressed previously (Underwood & Rausher, 2000; Bailey *et al.*, 2006; Tack *et al.*, 2010), and attempts have been made to link nonconsumptive effects of predators to the plant genotype the interaction occurs on (Thaler *et al.*, 2014; Kersch-Becker & Thaler, 2015). Traits of the herbivore altered by the nonconsumptive effect of a single predator type that have been investigated mostly include performance parameters like feeding, growth and body mass (Thaler *et al.*, 2014; Kersch-Becker & Thaler, 2015). Although per capita population growth and number of offspring in a population have been addressed (Kersch-Becker & Thaler, 2015), no attempts have been made to tease apart the contributions of consumptive and nonconsumptive effect on fitness of individual prey. Additionally, here we will investigate the individual and the combined effects of two very different predators on a shared prey and focus on key traits of reproductive behavior.

The first trait of the herbivore and its modulation by top-down and bottom-up effects we will investigate is clutch size. Extensive research on how females should adjust their clutch size to maximize fitness has been conducted for parasitoids (Jervis *et al.*, 2008; Rosenheim *et al.*, 2008; Wajnberg *et al.*, 2008). The clutch size of insect herbivores has also been well studied, and both bottom-up (Godfray, 1986; Pilson & Rausher, 1988; Kagata & Ohgushi, 2002) and top-down factors (Subinprasert & Svensson, 1988; Siemans & Johnson, 1992) have been shown to be important determinants. Assuming that herbivore females are not exclusively constrained by ecological pressures to maximize realized fecundity (Tatar, 1991; Clark & Faeth, 1998), females should place more eggs in the same clutch/ on the same plant to: i) increase the ability to overcome different plant defenses (Young & Moffett, 1979; Clark & Faeth, 1997), ii) take advantage of initial increased host plant suitability,

albeit followed by induced resistance (Fordyce, 2003), iii) lower the encounter probability of predators as increased time spent searching for prey leads to lower consumption (Vine, 1971). Such general mechanistic explanations serve as the basis for, e.g., the group defense hypothesis in insects (Denno & Benrey, 1997; Hunter, 2000). Besides the prey behavior, the behavior of how the predator is foraging on the herbivore eggs is determining if larger clutch sizes are advantageous. These different hunting modes (Miller *et al.*, 2014) of the predators can lead to increased survival chance in larger clutches if for example the predator is not consuming all encountered eggs immediately (Stephan *et al.* submitted). On the other hand placing too many eggs at one place will increase exploitative competition between the hatching larvae (Mitchell, 1975) and force them to migrate, which in turn may increase the predation risk (Matsumoto, 1990). In addition to changing the mean it can also be imagined that the frequency distribution of clutch sizes changes due to resource abundance (Kagata & Ohgushi, 2002) or predator protection (Atsatt, 1981). The frequency change may therefore also be important to understand the oviposition pattern of an herbivore in response to the host plant and predator presences. This change may especially be important if females modulate the distances between clutches on a plant to lower exploitative competition, with this modulation depending on the number of clutches (Stephan *et al.*, 2015). How herbivore clutch size, but especially clutch size distribution, is interactively affected by top-down and bottom-up effects have not been addressed and will be investigated here.

The second trait of the herbivore, and how it is affected by top-down and bottom-up effects we will investigate is oviposition rate. This trait can be a proxy for fitness and can change e.g. due to temperature (Tammaru *et al.*, 1996), plant species richness in the habitat (Unsicker *et al.*, 2010), or intraspecific exploitative competition (Hemptinne *et al.*, 1992). Oviposition rate can also be a measure of host plant acceptance and distinctions can be made between plant genotypes (Lehrman *et al.*, 2012). As fitness parameter it is possible to calculate the number of eggs not laid due to predator presence by comparing oviposition rate in predator presence and absence. Furthermore, by relating the number of not laid eggs to the number of predated eggs it is possible to calculate a consumptive:nonconsumptive effect ration (c:n-ration). Here we will use this ration to investigate how host plant suitability and predator type/combination interactively shape the contributions of consumptive and nonconsumptive effects on prey fitness.

The here utilized system is well studied and provides the following key components to study effects of plant genotypes, nonconsumptive effects and their

interaction. Genotypes of naturally hybridizing willows are differently suitable to the specialist leaf beetle *Phratora vulgatissima* and here we use four bred willow genotypes that are among the most suitable and unsuitable for the leaf beetle (Stenberg *et al.*, 2010). This beetle species is able to lower its oviposition rate according to how many conspecifics had visited the plant individual, while number of conspecific eggs seems of less important (Stephan *et al.*, 2015). At the same time the relative consumption of plant food and animal food (leaf beetle eggs) by two of the most important omnivorous predators changes in parallel with plant sap quality of genotypes. Because we can exclude plant defenses like trichomes to affect the omnivores (Björkman & Ahrne, 2005), we believe that predators are less motivated to forage for clutches if they can more easily satisfy their nutritional needs by consuming plant sap (Stenberg *et al.*, 2011a) (Stephan *et al.* submitted). These two predators also show distinctly different foraging modes (Björkman *et al.*, 2003). One exhibits a 'run and eat' behavior, meaning it does not consume all discovered eggs in a clutch which results in changed egg survival probabilities in differently sized egg clutches. Contrary, the other predator shows a 'find and stay' behavior and egg survival is independent of clutch size (Stephan *et al.* submitted). Therefore the aggregation behavior of the leaf beetle interacts with the foraging mode of the predator with this interaction also depending on the host plant quality because the strength of the underlying mechanism, attack-abatement, changes with quality of alternative food (plant sap) for the omnivore (Stephan *et al.* submitted). However, if and how the leaf beetles change their aggregation behavior due to predator presence has not been investigated.

We hypothesize that the leaf beetle females lay larger egg clutches and increase the proportion of larger clutches on unsuitable plant genotypes (1) and in presence of a predator, at least in the case where larger clutches are adventurous (2). We also hypothesize that predator presence will induce a lower oviposition rate as the female beetles try to minimize egg losses to predation (3). The lower oviposition rate will depend on predator species, and plant genotype, ultimately changing the contributions of consumptive and nonconsumptive effects to beetle fitness (changed c:n-ratio) (4).

Material and Methods

Study system

The adults and larvae of the herbivore *Phratora vulgatissima* L. (Coleoptera: Chrysomelidae), which has a wide distribution across Europe and Asia, skeletonize the leaves of its natural host plant, willows (*Salix* spp.). It is the most important specialist

herbivore of willow in Europe (Peacock & Herrick, 2000; Peacock *et al.*, 2002) and frequently reaches outbreak densities in natural willow stands and plantations (Björkman *et al.*, 2000a; Dalin *et al.*, 2009). In willow plantations used for biomass production, outbreaks can reduce growth by up to 40% (Björkman *et al.*, 2000b). Adults overwinter in shelter-providing vertical objects, for example reeds or trees with ageing bark (Björkman & Eklund, 2006). They emerge in April, feed for about two weeks, mate and subsequently lay hundreds of eggs on the underside of leaves in clutches of 1–50 eggs. Larvae hatch after 15–20 days, feed gregariously on leaves during the first and second instar, and then solitarily during the third instar (Kendall *et al.*, 1996). Larvae pupate in the soil; adults emerge in August and after a short period of feeding, find hibernation sites.

Anthocoris nemorum L. (Heteroptera: Anthocoridae) is considered an important predator against *P. vulgatissima* because it can consume large numbers of eggs (Björkman *et al.*, 2003) and is an effective biological control agent in apple orchards (Sigsgaard, 2010). Like most other predatory heteropteran bugs, *A. nemorum* also feeds on shallowly located fluids from the green parts of host plants. However, it is mainly regarded as a predator (Lauenstein, 1979).

Orthotylus marginalis Reut. (Heteroptera: Miridae) also consumes large numbers of *P. vulgatissima* eggs (Björkman *et al.*, 2003) and was observed to be mainly predacious (Lehman, 1932), while other observations supported the impression that it can survive on a minimal amount of animal food but that it has a preference for such food (Kullenberg, 1944).

Beetles and bugs were collected from natural populations in the Uppsala region of Sweden. The *Salix* genotypes (*S. viminalis*: 78021 and 78183; *S. dasyclados*: Gudrun and Loden) selected for the experiments were chosen because they differ in chemical composition and have previously been tested for their suitability for both the leaf beetle and the omnivorous predators. The suitability of these genotypes for the leaf beetle has been found to increase in the order Gudrun < Loden < 78021 < 78183 (Stenberg *et al.*, 2010), whereas the suitability for the omnivorous bug in the absence of prey follows the reverse order. When prey is present, the most suitability of these genotypes for the omnivore is genotype 78183, whereas the suitability of the genotypes Gudrun, Loden and 78021 are similar (Stenberg *et al.*, 2011a).

Clones of the different genotypes were grown in the greenhouse where experiments were performed (23°C, RH 80, L18:D6). Plants had between 17 and 35 leaves and were randomly selected for different experiments and treatments. At least one day before each experiment, plants were prepared by removing the top

Table 1: Analysis-of-deviance tables (Type III test) from generalized linear mixed models investigating the oviposition behaviour of the leaf beetle *Phratora vulgatissima* and how it is altered by the presence of different predators and on what genotype the interaction occurred on. Genotype = plant genotype; Treatment = always two leaf beetles with varying combination and number of predators; Eggs laid = cumulative number of eggs on a plant. Non-significant terms (italicized) were removed stepwise from the final model starting from the bottom row. “/” means “nested within”; Obs = each observation

Model	Model Type	Random factor	Response Variable	Explanatory Variables	X ²	Df	AIC	R ² _{GLMM(m)}	R ² _{GLMM(c)}	p-value
Does the mean clutch size depend on the predator treatment (Control, 2 AN) or the plant genotype (Gudrun, Loden, 78021, 78183)?										
M1	GLMM (Poisson)	Plant/ Obs	Clutch size	intercept	160.83	1	2799.12	0.28	0.33	<0.001
				Genotype	42.69	3	2799.12	0.28	0.33	<0.001
				Treatment	2.66	1	2799.12	0.28	0.33	0.10
				Eggs laid	1.20	1	2799.12	0.28	0.33	0.27
				Treatment × Eggs laid	5.07	1	2799.12	0.28	0.33	0.02
				<i>Genotype × Eggs laid</i>	4.83	3	2800.29	0.29	0.33	0.18
				<i>Genotype × Treatment</i>	4.20	3	2802.17	0.30	0.34	0.23
Does the mean clutch size depend on the predator treatment (2 OM/1 AN + 1OM/4 OM) or the plant genotype (Loden, 78183)?										
M2	GLMM (Poisson)	Plant/ Obs	Clutch size	intercept	286.40	1	1347.03	0.11	0.12	<0.001
				Genotype	0.60	1	1347.03	0.11	0.12	0.43
				Treatment	3.49	2	1347.03	0.11	0.12	0.17
				Eggs laid	14.94	1	1347.03	0.11	0.12	<0.001
				<i>Genotype × Eggs laid</i>	3.36	1	1345.74	0.12	0.13	0.06
				<i>Treatment × Eggs laid</i>	3.59	1	1345.77	0.14	0.14	0.16
				<i>Genotype × Treatment</i>	0.86	2	1348.88	0.14	0.14	0.64
Does the eggs laid per plant depend on the predator treatment (Control, 2 AN) or the plant genotype (Gudrun, Loden, 78021, 78183)?										
M3	GLMM (Poisson)	Plant	Eggs laid	intercept	955.53	1	657.50	0.77	0.77	<0.001
				Genotype	190.43	3	657.50	0.77	0.77	<0.001
				Treatment	11.35	1	657.50	0.77	0.77	<0.001
				<i>Genotype × Treatment</i>	4.24	3	659.38	0.78	0.78	0.23
Does the eggs laid per plant depend on the predator treatment (2 OM/1 AN + 1OM/4 OM) or the plant genotype (Loden, 78183)?										
M4	GLMM (Poisson)	Plant	Eggs laid	intercept	557.70	1	408.42	0.51	0.51	<0.001
				Genotype	45.72	1	408.42	0.51	0.51	<0.001
				Treatment	1.21	2	411.22	0.52	0.52	0.54
				<i>Genotype × Treatment</i>	0.46	2	414.75	0.53	0.53	0.79
Does the survival within clutches depend on the clutch size and the plant genotype (Gudrun, Loden, 78021, 78183)?										
M5	GLMM (Binomial)	Plant/ Obs	Survival (survived/ predated)	intercept	0.05	1	522.41	0.25	0.29	0.81
				Clutch size	1.18	1	522.41	0.25	0.29	0.27
				Genotype	2.33	3	522.41	0.25	0.29	0.50
				Clutch size × Genotype	8.89	3	522.41	0.25	0.29	0.03
Does the survival within clutches depend on the predator treatment and the clutch size the plant genotype (Loden, 78183)?										
M6	GLMM (Binomial)	Plant/ Obs	Survival (survived/ predated)	intercept	13.40	1	317.29	0.08	0.11	<0.001
				Clutch size	0.02	1	317.29	0.08	0.11	0.86
				Genotype	3.73	1	317.29	0.08	0.11	0.05
				Treatment	3.35	2	317.29	0.08	0.11	0.18
				Clutch size × Treatment	11.59	2	317.29	0.08	0.11	<0.01
				<i>Genotype × Treatment</i>	4.06	2	343.68	0.37	0.40	0.13
				<i>Clutch size × Genotype</i>	0.08	1	332.96	0.38	0.38	0.76
<i>Clutch size × Genotype × Treatment</i>	2.90	2	346.52	0.61	0.62	0.23				
Does the survival of eggs on a plant depend on the total number of eggs on the plant and the plant genotype (Gudrun, Loden, 78021, 78183)?										
M7	GLMM (Binomial)	Plant/ Obs	Survival (survived/ predated)	intercept	6.64	1	198.73	0.25	0.25	<0.01
				Genotype	18.58	3	198.73	0.25	0.25	<0.001
				Eggs on plant	2.46	1	198.50	0.28	0.28	0.11
				<i>Eggs on plant × Genotype</i>	3.18	3	201.22	0.31	0.31	0.36
Does the survival of eggs on a plant depend on the total number of eggs on the plant, the predator treatment and the plant genotype (Loden, 78183)?										
M8	GLMM (Binomial)	Plant/ Obs	Survival (survived/ predated)	intercept	32.72	1	223.21	0.28	0.28	<0.001
				Genotype	13.38	1	223.21	0.28	0.28	<0.001
				Eggs on plant	1.78	1	223.37	0.30	0.30	0.18
				Treatment	4.43	2	222.93	0.37	0.37	0.10
				<i>Eggs on plant × Treatment</i>	0.12	2	226.80	0.36	0.36	0.93

2-4 newly emerged, incompletely unfolded leaves and the lowest old and withering leaves to standardize shoots. All plants had approximately the same cumulative leaf area because smaller leaves (see (Stephan *et al.*, 2015)) were compensated by higher number of leaves on a plant.

Oviposition in presence and absence of different predators

We investigated the combined effect of different *Salix* genotypes and the presence of either of the two predators singly or together on the egg-laying behavior of the leaf beetle. Ovipositing females of *P. vulgatissima* were allowed to lay eggs for six days on the prepared plants. Five treatments, with at least ten replicates (plant individuals) for a treatment-genotype combination, were used: 1) only two leaf beetles (Control); 2) two leaf beetles and two *A. nemorum* predators (2 AN); 3) two leaf beetles and two *O. marginalis* predators (2 OM); 4) two leaf beetles, one *A. nemorum*, and one *O. marginalis* predators (1 AN 1 OM); and 5) two leaf beetles and four *O. marginalis* predators (4 OM). Treatment one and two were performed in 2009 (experimental part one) while the remaining treatments were done in 2015 (experimental part two) under the same conditions in the green house. However, due to the late development of *A. nemorum* adults in 2015 the nymphs of *O. marginalis* were already third to fourth stage, instead of the first and second as in 2009. The eggs in each clutch were counted and the total number of eggs per plant calculated. Both predators have a sucking feeding behavior and leave empty egg shells behind, that we counted and calculated the proportion of eggs surviving per clutch.

Calculation of nonconsumptive effect on reproductive output

We were interested in the contributions of consumptive and nonconsumptive effects on the total number of surviving eggs. In order to do so we used the number of eggs laid on a plant genotype in absence of any predator as reference and compared it to the number of eggs laid in predator presence. We used the proportion of consumed eggs (number of eggs predated with predator / number of eggs laid with predator) to calculate the consumptive effect and the proportion of not laid eggs due to predator presence (number of eggs laid with predator / number of eggs laid without predator) to calculate the nonconsumptive effect. We expressed the results as a consumptive:nonconsumptive ratio (c:nc-ratio). In contrast to the statistical analysis these comparisons were done using the control from the experimental part one to calculate the contributions for the predator

treatments in part two which we think is valid as ratios of ratios are compared.

Data analysis

The count and survival data were analyzed with generalized linear mixed models with the plant individual as random effect. We also included a random effect for every observation nested within the plant to account for possible model overdispersion. For the proportion of survival within clutches and within all eggs laid on a plant (predated/survived) we used a binomial distribution with logit link and for the count data (clutch size, eggs on plant) a Poisson distribution with a log link function. Because both experimental parts were not performed in the same year we did not (feel confident to) pool these data and modelled these experimental parts separately. In addition to the effect of the plant genotype and the predator treatments on the mean clutch size we were also interested in the effect on the clutch size distribution. Therefore, we included the number of eggs laid on a plant in the models investigating the clutch size and compared the slopes. Due to very few data for genotypes with low acceptance we did not start with a model including the interaction between eggs laid, treatment, and plant genotype but with only 2-way interactions. Besides investigating the change in the mean clutch size with models that assign Poisson distributions to every count observation we were also interested if the presence of the predators or the different plant genotypes have any effect on the actual distribution of clutch sizes. We therefore performed Kolmogorov-Smirnov tests (KS-test) an illustrated the point of maximum separation (D-values) between both distributions as measure of how different distributions are. Because these comparisons uses the relative distribution of the data and are therefore independent of the number of eggs laid (that may vary between the years and therefore experimental parts) we here related the distributions of all predator treatments to the control.

Results

The predator treatments had no effect on the mean clutch size of *P. vulgatissima*, but the plant genotype was strongly modulating the mean clutch size (Tab. 1 M1, M2) with the *S. viminalis* genotypes 78021 leading to largest clutches (Fig. 1a) in presence of *A. nemorum* in the first part of the experiment. In the second part of the experiment the mean clutch size did not differ among treatments but seemed to resemble the genotype specific size from the first part. In general clutches became larger if more eggs were laid on a plant with this relationship increasing stronger in the presence of the predator in the first part (Fig. S1) and

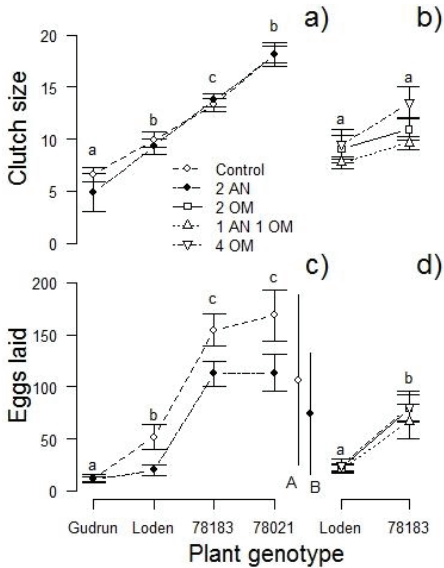


Figure 1: Mean (\pm SE) clutch size and eggs laid on individual plants by two *Phratora vulgatissima* females on four different *Salix* genotype (*S. dasyclados*: Gudrun, Loden; *S. viminalis*: 78183, 78021) and the presence of predators treatment (Control = only leaf beetles, AN = *Anthocoris nemorum*, OM = *Orthotylus marginalis*) for the first (a, c) and second (b, d) experimental part. Lowercase letters indicate differences between genotypes and upper case letters differences between overall means (\pm SD) of treatments ($p < 0.05$; Tukey contrast).

tend to become stronger on the plant genotype Loden compared to 78183 in the second part (Fig. S2). The overall mean (SD) clutch size without predator was 14.0 (7.5), with two *A. nemorum* 14.1 (7.2), with two *O. marginalis* 10.5 (7.6), with one *O. marginalis* and one *A. nemorum* 9.1 (4.3), and with four *O. marginalis* 12.1 (8.8) eggs per clutch. Most eggs were laid on the *S. viminalis* genotypes (part one: 78183, 78021; part two: 78183) and more eggs in presence of *A. nemorum* compared to predator absence (Tab. 1 M3; Fig. 1c) while the remaining predator treatments had similar effects on the number of eggs laid (Tab. 1 M4; Fig. 1d).

Taking a closer look at the actual distributions showed that the presence of *A. nemorum* changed the frequency of clutch sizes leading to less variable and smaller clutch sizes in predator presence compared to predator absence for the same cumulative fraction (e.g.: clutch size a fraction 0.5: Control: 17.5; 2 AN: 6.5). In fact this was true for all predator treatments (Fig. 2a, c; p-values (Bonferroni-Holm corrected) / D-values: Control vs 2 AN: $<0.001 / 0.71$, Control vs 2 OM: $<0.001 / 0.38$, Control vs 1 AN 1 OM: $<0.01 / 0.25$, Control vs 4 OM: $<0.05 / 0.19$). The largest differences was found for *A. nemorum* where in the

predator present treatment 92 % of the clutches are smaller than the point of maximum separation while only 21% are smaller in the predator absent treatment. The clutch size distribution was also specific for each genotype (Fig. 2b, d) with decreasing variability and size for the same cumulative fraction from genotype 78021 to Gudrun in the first part of the experiment (p-values (Bonferroni-Holm corrected) / D-values: 78021 vs 78183: $<0.001 / 0.28$; 78021 vs Loden: $<0.001 / 0.55$; 78021 vs Gudrun: $<0.001 / 0.77$; 78183 vs Loden: $<0.001 / 0.32$; Loden vs Gudrun: $<0.01 / 0.44$) while in the second part the distributions tend to differ between 78183 and Loden (p-values / D-values: $=0.07 / 0.20$).

The egg survival in clutches (Tab.1 M5) and the survival of all eggs on a plant (Tab.1 M7) in presence of the predator *A. nemorum* were depending on the plant genotype with lower survival on Loden (Fig. 3). Egg survival was generally lower in the second part of the experiment and not different between treatments (Fig. 4a-c) although, again, higher for 78183 than for Loden (per clutch and per eggs on plant; Fig. 4d). For the second part of the experiment we found a tendency of plant genotype having an effect on the egg survival in clutches and a significant interaction between clutch size and treatment (Tab. 1 M6). This interaction, and the interaction between clutch size and genotype in the first part (Tab. 1 M5), can be attributed to survival increasing with clutch size for the 1 AN 1 OM-treatment (Fig. 4) and for the genotypes 78183 and 78021 (Fig. 3), respectively. Egg survival was generally lower in the second part of the experiment which we attribute to the before mentioned usage of third instars of *O. marginalis*.

Visualizing the consumptive and nonconsumptive effects for all predator treatments by using the number of eggs laid on a plant from the first part of the experiment revealed that the plant genotype altered not only the consumptive effect (egg survival) but also the nonconsumptive effect (eggs not laid due to predator presence) (Fig. 5). The nonconsumptive effect ranged from 0.1 (= 10% less eggs laid in predator presence) to 0.62 and was generally larger on genotype Loden than on genotype 78183. The c:nc-ratio ranged from 0.29 indicating an around three times as stronger nonconsumptive than consumptive effect, over exactly the same contributions with a ratio of 1, to a more than two and a half times stronger consumptive than nonconsumptive effect (2.67).

Discussion

In line with our first hypothesis we found that the mean clutch size of *P. vulgatissima* is driven by plant genotype with clutch size increasing with host plant suitability. We were able to detect the increase despite the fact that clutch sizes generally become larger with

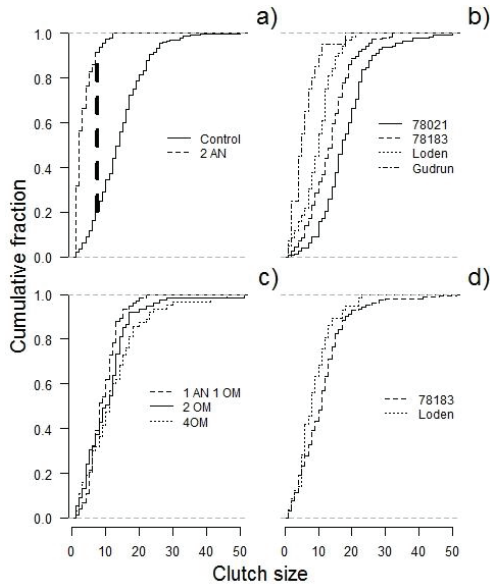


Figure 2: Cumulative fraction plot showing the relative distribution of clutch sizes from the first (a, b) and second part (c, d) of the experiment (Treatments: Control = only leaf beetles, AN = *Anthocoris nemorum*, OM = *Orthotylus marginalis*; Plant genotypes: 78183, 78021, Loden, Gudrun). All treatments show smaller clutch sizes in predator presence compared to predator absence and decreasing egg clutch size from genotype 78021 to genotype Gudrun (b) for the same cumulative fraction. Clutch sizes tend to be larger on genotype 78183 than on Loden in the second part. The bold broken vertical line indicates exemplary the point of maximal separation D (KS-test).

the number of herbivore eggs laid on a plant. In contrast (Janz & Thompson, 2002), but similar (Pilson & Rausher, 1988) to studies on Lepidoptera, our results suggest that *P. vulgatissima* adjusts its clutch size according to the suitability of the plant (genotype), with larger egg clutches on the most suitable plant genotypes 78183 and 78021 than on the relatively resistant genotypes Gudrun and Loden. We showed previously that this leaf beetle increases its distances between clutches on a plant due to larger leaf area of the unsuitable genotype, not the unsuitability itself (Stephan *et al.*, 2015). By contrast, here we observed that, although larger leaves, the unsuitable genotypes (Loden, Gudrun) received considerably smaller herbivore egg clutches and showed that the genotype specific mean clutch size depends on plant suitability - not leaf area - as shown in other studies (Kagata & Ohgushi, 2002). As with the increase of distances between clutches the adaptive mechanism for modulating clutch size would be to avoid intraspecific exploitative competition, because the larvae feed

gregariously close to the site of hatching until the 3rd instar and only then start to move to other plant parts. Competition should be lower on suitable plant genotypes, which can support more larvae in an equivalent feeding area. This expectation of lower competition is supported by theoretical considerations (Pilson & Rausher, 1988; Roitberg *et al.*, 1999), as well as by experimental studies of other herbivorous insects (Freese & Zwölfer, 1996). The beetle females therefore match the number of larvae hatching from an egg clutch to the food quality to reduce the risk of larval aggregation problems. Besides comparing the mean clutch size we also found changes in the variability and median. Herbivore egg clutch size distribution changes have been found for the leaf mining moth *Paraleucoptera sinuella*, that decreased median and variability on smaller *Salix miyabeana* leaves compared to larger *Populus sieboldii* leaves (Kagata & Ohgushi, 2002). We showed here that not only interspecific differences in leaf area, but also suitability can explain modulation of this trait because lower variation and median were found on suitable, but larger leaves. The lowering of intraspecific competition is therefore achieved by laying generally smaller, but also more smaller clutches.

Contrary to our expectation the beetle females did not respond to predator presence by changing their mean clutch size. However, the increase of egg clutch size with number of eggs laid on a plant was stronger in presence as compared to absence of *A. nemorum*, which indicates that ovipositing leaf beetles respond to the predators. In investigating the actual distribution we could see how either of the predator types/combinations lowered the median and the variation in clutch size and clutch size was always smaller for the same cumulative fraction. This means that, although beetle females that perceive a predator have a lower oviposition rate associated with smaller clutch size, we could still observe that more smaller clutches were laid compared to predator absence (relative to the specific variation in clutch size). Although changes in mean clutch size due to predator presence have been reported previously for a moth species (Subinprasert & Svensson, 1988), we did not find any study investigating changes in distribution of clutch sizes. We only found the mistletoe butterfly *Ogyris amaryllis* (Lyeaenidae) to lay more eggs in a clutch if (aphids-, scale-, and leafhoppers-) tending ants on these mistletoes were present (Atsatt, 1981). The larger clutch sizes came along with a shift from a clutch size variation between 1 and 3 to 1 and 24. The adaptive mechanism apparently is an increased indirect predator protection as the ants do not feed on the eggs but guard the mistletoe. In our system, the investigated leaf beetle seems to respond differently with increasing the number of small clutches, which is especially

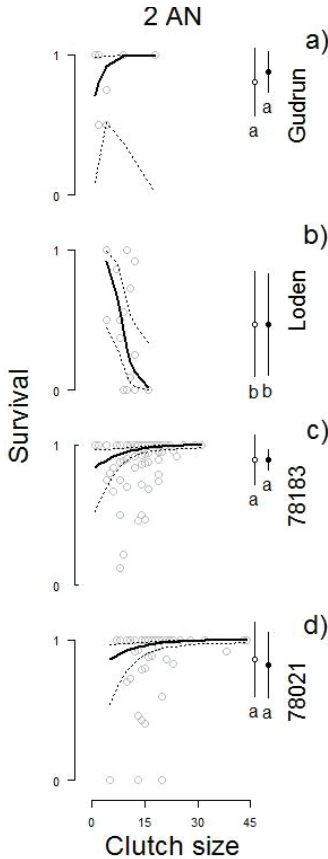


Figure 3: Individual egg survival within clutches in presence of the predator *Anthocoris nemorum* in relation to clutch size for all four plant genotypes (*Salix dasyclados*: Gudrun, Loden; *Salix viminalis*: 78021, 78183). Grey circles show the proportion survived eggs within clutch, and the lines indicate the model predictions with bootstrapped confidence limits. Black circles on the sides show the mean (\pm SD) survival per clutch (open) and per cumulative number of eggs on a plant (closed) for each plant genotype. Letters indicate differences between genotypes ($p < 0.05$; Tukey contrast).

surprising as we showed that (artificially manipulated, Stephan et. al. submitted) and natural laid (this study) larger clutch size increase survival in the case of *A. nemorum*, even if only one of both predators is *A. nemorum*. The 'find and stay' behaviour of *O. marginalis*, however, represents a numerical dilution effect of zero leading to clutch size independent survival (Stephan et. al. submitted). We now validated that the attack-abatement mechanism is acting in a less artificial set up and natural variation of clutch sizes and increases individual eggs survival in a clutch and found more support for these functional differences between

the predators. Nevertheless, the question remains why the leaf beetles do not increase egg clutch size, at least in the presence of *A. nemorum*? Both predators and their combination lead to more smaller clutches indicating that the leaf beetles may not discriminate between the predators. Though, we also investigated the vertical position of where on the shoot the leaf beetles laid their eggs and where on the shoots each predator is preferentially foraging. Because including these results here would have been too extensive these questions will be addressed elsewhere (Stephan et. al. manuscript), but show that the predators have different habitat domains. The ovipositing leaf beetles avoid them, meaning the main response would not be modulation of clutch size but avoiding the area where predators are perceived in the first place.

Laying fewer eggs on unsuitable plant genotypes and in predator presence than absence is another behavioural response that may be more important for egg survival than clutch size modulation. In our study we neither evaluated the oviposition choice in a field set up (Tschanz et al., 2005) nor with alternative host plants but with a no-choice assay. However, the oviposition rate is a good proxy for life time fitness in this species and our results supported previous findings that lower numbers of eggs are laid by individual females on less suitable plant genotypes (Stenberg et al., 2010; Lehrman et al., 2012). Because the cumulative leaf area of the genotypes was similar we can also conclude that host plant acceptance was due to suitability not larger leaf/feeding area. More of interest here was the finding that predator presence also lowers oviposition rate, which makes oviposition rate modulation as the a key behavioral response in this species depending on aggregation level on the plant (Stephan et al., 2015), host plant suitability and predators. In the presence of *A. nemorum* the oviposition rate was generally lower compared to the predator absence treatment. This pattern also seems to reoccur in similar strength in the second part of the experiment for *O. marginalis* at density of two and four and in the treatment with one *Orthotylus marginalis* and one *A. nemorum*. Predatory mite eggs (only nonconsumptive effect) can trigger lower oviposition rates in herbivorous insects (Walzer & Schausberger, 2009) and presence, but not indirect cues, of an intraguild predatory mite can trigger egg retention of a phytoseiid mite (Montserrat et al., 2007). Predator presence seemed to lower population growth of aphids (Nelson et al., 2004; Nelson, 2007; Kersch-Becker & Thaler, 2015), but our results seems to be the first report of predators lowering oviposition rate of individual herbivorous insects. The lowered oviposition rate probably represents the most direct measure of a nonconsumptive effect on fitness and was achieved by relating how many eggs are laid in

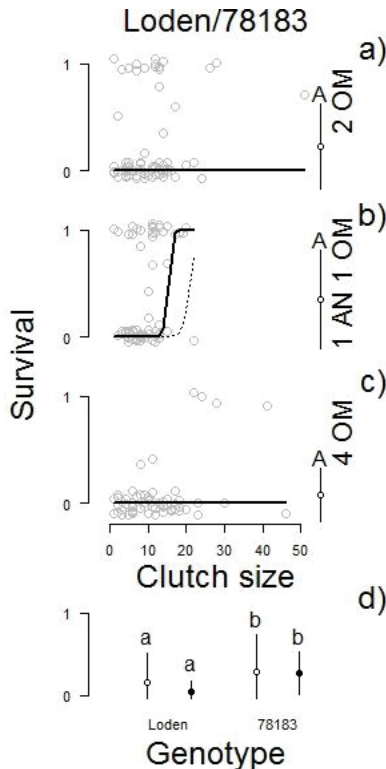


Figure 4: Individual egg survival within clutches in relation clutch size for both plant genotypes (*Salix dasyclados*: Loden; *Salix viminalis*: 78183) and predator treatments (AN = *A. nemorum*, OM = *O. marginalis*). Proportion surviving beetle eggs is jittered to increase visibility and the lines show the model prediction with bootstrapped confidence limits. The lowest figure (d) shows the overall mean of all tree treatments. Black circles show the mean (\pm SD) survival per clutch (open) and per cumulative number of eggs on a plant (closed). Capital letters indicate differences between treatments and lowercase letters differences between genotypes ($p < 0.05$; Tukey contrast).

absence/presence on different plant genotypes (that offer different food quality for the omnivorous predator) to the number of eggs predated; which we expressed as c:n-c-ratio. In doing so we found that the strength of the nonconsumptive effect ranged from at least being one third up to two and a half times as strong as the consumptive effect, depending on plant genotype. In other words, the presence of the predator was lowering the oviposition rate by at least one third compared to its egg consumption. How many eggs were not laid in relation to how many were consumed also depended on the predator type. In both cases were *A. nemorum* was present the consumptive and the nonconsumptive effect were larger on Loden compared

to 78183. We believe the higher consumption is due to lower quality food from the plant and the higher nonconsumptive effect is a result of increased encounter with ovipositing females during more intense foraging. However, the relative strength of nonconsumptive effect compared to the consumptive effect was lower (higher c:n-c-ratio) on Loden than the remaining genotypes meaning *A. nemorum* presence was not that disturbing for the females on Loden. Increased time spent on egg consumption is therefore accompanied with less time spent searching for prey and thus disturbing the ovipositing leaf beetle females less. Consequently, although the plant gains protection through egg consumption there might be also a “cost” in form of lower benefit from the nonconsumptive effect.

Another important implication from this study is that the question if an omnivorous predator could act as an indirect defense of a plant/genotype should be addressed by including the herbivore and its behavior in the experiment. With a more artificial set up (pinning clutches on leaves) we previously found lower egg consumption on plant genotype Loden as compared to genotypes 78183 and 78021 causing doubt on the bodyguard function of *A. nemorum* (Stenberg *et al.*, 2011b). Contrary to these previous results, in both parts of our experiments egg survival was lower on the plant genotype Loden at both scales (clutches, eggs on plant). We showed previously in olfactometric assays that a *S. dasyclados* genotype similar to genotype Loden is more attractive to *A. nemorum* than a *S. viminalis* genotype similar to 78183 and 78021, but only if the plants were attacked by the leaf beetle. Otherwise, the two plant genotypes had a similar attractiveness, which was only slightly higher than that of ambient air (Lehrman *et al.*, 2013). Therefore, including the herbivore would not only include the intrinsic sap quality but also foraging kairomones from beetles (Fernandez & Hilker, 2007) or plant volatile induction due to feeding or oviposition (Dicke & Baldwin, 2010) that may change the predator behavior. These behavioral changes are probably also predator specific as we saw that for *O. marginalis* the consumptive and nonconsumptive effects were again stronger on genotype Loden than genotype 78183, but the c:n-c-ratio was higher on genotype 78183 meaning presence of this predator was here not that disturbing to the beetle females. Also, increasing the density of *O. marginalis* seems to even out differences between genotypes. In many biological control strategies the estimation of pest density is done by counting the pest individuals or estimating the damage to the crop and relating it to the predator/parasitoid density/diversity under consideration of spill-over and dilution effects (Andow, 1991; Stephan *et al.*, 2016). Yet, after accounting for these effects care should be taken to directly link any desired pest control to consumption as

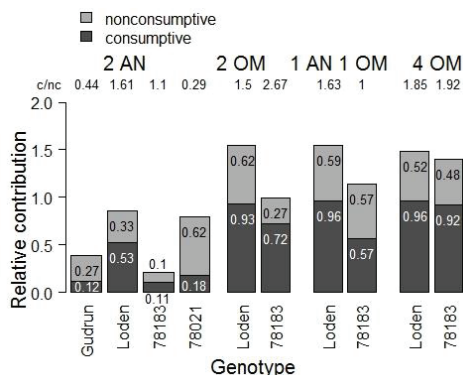


Figure 5: Contributions of consumptive and nonconsumptive effect to survival of herbivore eggs depending on predator treatment (AN = *Anthocoris nemorum*, OM = *Orthotylus marginalis*) and plant genotype (*S. dasyclados*: Gudrun, Loden; *S. viminalis*: 78021, 78183). The consumptive effect is expressed as the proportion of predated eggs of the total number of laid eggs on a plant and the nonconsumptive effect as the proportion of eggs not laid in predator presence compared to the predator absence treatment (numbers within bars, respectively). The numbers above the bars express the consumptive:nonconsumptive-ratio (for each genotype-treatment combination).

there might be a nonconsumptive effect component in form of egg retention also. Although it is probably difficult to detect if predation risk would be lower on specific plant genotypes in the field (Stephan *et al.*, 2016) we found first evidence that *P. vulgaticissima* could do so. Certainly the influence of retaining or delaying eggs still needs to be evaluated as many other factors like habitat heterogeneity (Andersson *et al.*, 2013), valuing own performance higher than that of the offspring (Mayhew, 2001), or higher predation risk on otherwise suitable hosts (Egusa *et al.*, 2008) could override these positive effect on egg survival. Anyhow, the concept of indirect defense and its application as bio-control strategy would therefore gain in providing an estimate of not only the consumptive, but also the nonconsumptive effect of different predators on different host plants.

Recently Kersch-Becker and Kaplan elucidated the interaction of consumptive, nonconsumptive effects and host plant resistance in using genetically modified tomato lines and predators with impaired mandibles (Kersch-Becker & Thaler, 2015). With more natural host plants (we used commercial clones, but *Salix* naturally hybridize) and unharmed predators we could show how oviposition rate and clutch size of the leaf beetle *P. vulgaticissima* change due to quality of different host plants and the presence of predators that show different foraging behavior. Increasing clutch size in response to predator presence in cases where it would benefit egg survival does not occur, but other

behaviors may be more important. Because of the strength of at least a third compared to the consumption and the interaction between bottom-up and top-down effects we argue to increase awareness of nonconsumptive effects in biocontrol strategies. The merits of using direct than rather indirect measures for fitness consequences included directly relating consumptive and nonconsumptive effects to the specific predator/predator combination and the specific plant genotype the interaction occurred on. Because oviposition rate and clutch size are key life history traits for reproduction the understanding of their modulation by bottom-up and top-down effects will help to understand how and why species aggregate.

Acknowledgements

We thank Karin Eklund and Sussi Björkman for their assistance during the experiment and Mikael Andersson Franko for inspiring discussions about the statistical analysis. This study was funded by the Swedish Energy Agency (Energimyndigheten) and the Swedish Research Agency Formas.

References

- Agrawal, A. a (2007). Macroevolution of plant defense strategies. *Trends in ecology & evolution*, 22(2), pp 103–9.
- Andersson, P., Löfstedt, C. & Hambäck, P. a. (2013). Insect density-plant density relationships: A modified view of insect responses to resource concentrations. *Oecologia*, 173(4), pp 1333–1344.
- Andow, D. (1991). Vegetational diversity and arthropod population response. *Annual Review of entomology*, 36, pp 561–586.
- Atsatt, P. R. (1981). Ant-dependent food plant selection by the mistletoe butterfly *Ogyris amaryllis* (Lycaenidae). *Oecologia*, 48, pp 60–63.
- Bailey, J. K., Wooley, S. C., Lindroth, R. L. & Whitham, T. G. (2006). Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecology letters*, 9(1), pp 78–85.
- Beckerman, A. P., Uriarte, M. & Schmitz, O. J. (1997). Experimental evidence for a behavior-mediated trophic cascade in a terrestrial food chain. *Proceedings of the National Academy of Sciences of the United States of America*, 94(20), pp 10735–10738 The National Academy of Sciences of the USA.
- Björkman, C. & Ahrne, K. (2005). Influence of leaf trichome density on the efficiency of two polyphagous insect predators. *Entomologia*

- Experimentalis et Applicata*, 115(1), pp 179–186.
- Björkman, C., Bengtsson, B. & Hågström, H. (2000a). Localized outbreak of a willow leaf beetle: plant vigor or natural enemies? *Population Ecology*, 42, pp 91–96.
- Björkman, C., Dalin, P. & Eklund, K. (2003). Generalist natural enemies of a willow leaf beetle (*Phratora vulgatissima*): abundance and feeding habits. *Journal of Insect Behavior*, 16(6), pp 747–764.
- Björkman, C. & Eklund, K. (2006). Factors affecting willow leaf beetles (*Phratora vulgatissima*) when selecting overwintering sites. *Agricultural and Forest Entomology*, 8(2), pp 97–101.
- Björkman, C., Höglund, S., Eklund, K. & Larsson, S. (2000b). Effects of leaf beetle damage on stem wood production in coppicing willow. *Agricultural and Forest Entomology*, 2(2), pp 131–139 Wiley Online Library.
- Clark, B. & Faeth, S. (1997). The consequences of larval aggregation in the butterfly *Chlosyne lacinia*. *Ecological Entomology*, 22(4), pp 408–415.
- Clark, B. & Faeth, S. (1998). The evolution of egg clustering in butterflies: a test of the egg desiccation hypothesis. *Evolutionary Ecology*, pp 543–552.
- Creel, S., Winnie, J. a & Christianson, D. (2009). Glucocorticoid stress hormones and the effect of predation risk on elk reproduction. *Proceedings of the National Academy of Sciences of the United States of America*, 106(30), pp 12388–93.
- Dalin, P., Kindvall, O. & Björkman, C. (2009). Reduced population control of an insect pest in managed willow monocultures. *PLoS one*, 4(5), p e5487.
- Degen, T., Dillmann, C., Marion-Poll, F. & Turlings, T. C. J. (2004). High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. *Plant physiology*, 135(4), pp 1928–38.
- Denno, R. & Benrey, B. (1997). Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. *Ecological Entomology*, 22(2), pp 133–141 Wiley Online Library.
- Denno, R. F., Gratton, C., Peterson, M. & Langelotto, G. (2002). Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. *Ecology*, 83(5), pp 1443–1458.
- Dicke, M. & Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the "cry for help". *Trends in Plant Science*, 15(3), pp 167–75 Elsevier Ltd.
- Egusa, S., Nishida, T., Sawada, H. & Fujisaki, K. (2008). Is selection of host plants by *Plagioderaversicolora* based on plant-related performance? *Entomologia Experimentalis et Applicata*, 128(2), pp 258–264.
- Fernandez, P. & Hilker, M. (2007). Host plant location by Chrysomelidae. *Basic and Applied Ecology*, 8(2), pp 97–116.
- Fordyce, J. a (2003). Aggregative feeding of pipevine swallowtail larvae enhances hostplant suitability. *Oecologia*, 135(2), pp 250–7.
- Freese, G. & Zwölfer, H. (1996). The problem of optimal clutch size in a tritrophic system: the oviposition strategy of the thistle gallfly *Urophora cardui* (Diptera, Tephritidae). *Oecologia*, 108(2), pp 293–302.
- Godfray, H. C. J. (1986). Clutch size in a leaf-mining fly (*Pegomyza nigrotarsis*: Anthomyiidae). *Ecological Entomology*, 11, pp 75–81.
- Harfenist, A. & Ydenberg, R. (1995). Parental provisioning and predation risk in rhinoceros auklets (*Cerorhinca monocerata*): effects on nestling growth and fledging. *Behavioral Ecology*, 6(1), pp 82–86.
- Hemptonne, J. L., Dixon, A. F. G. & Coffin, J. (1992). Attack strategy of ladybird beetles (Coccinellidae): factors shaping their numerical response. *Oecologia*, 90(2), pp 238–245.
- Hunter, a F. (2000). Gregariousness and repellent defences in the survival of phytophagous insects. *Oikos*, 91(2), pp 213–224.
- Janz, N. & Thompson, J. (2002). Plant polyploidy and host expansion in an insect herbivore. *Oecologia*, 130(4), pp 570–575.
- Jervis, M. a, Eilers, J. & Harvey, P. H. (2008). Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. *Annual Review of Entomology*, 53, pp 361–85.
- Kagata, H. & Ohgushi, T. (2002). Clutch size adjustment of a leaf-mining moth (Lyonetiidae: Lepidoptera) in response to resource availability. *Ecology and Population Biology*, 95(2), pp 213–217.
- Kaplan, I. & Thaler, J. S. (2010). Plant resistance attenuates the consumptive and non-consumptive impacts of predators on prey. *Oikos*, 119(7), pp 1105–1113.
- Karban, R. (2011). The ecology and evolution of induced resistance against herbivores.

- Functional Ecology*, 25(2), pp 339–347.
- Kendall, D., Wiltshire, C. & Butcher, M. (1996). *Phenology and population dynamics of willow beetles (Coleoptera; Chrysomelidae) in short-rotation coppiced willows at Long Ashton. ETSU (DTI) Biofuels Study B/M4/00487/14/REP. IACR-Long Ashton Research Station.*
- Kersch-Becker, M. F. & Thaler, J. S. (2015). Plant resistance reduces the strength of consumptive and non-consumptive effects of predators on aphids. *Journal of Animal Ecology*, pp 1222–1232.
- Kullenberg, B. (1944). Studien über die Biologie der Capsiden. *Zoologiska Bidrag från Uppsala*, 23, pp 240–243 Uppsala: Almqvist & Wiksellis Boktryeri AB.
- Lauenstein, G. (1979). Zur Aufnahme von Pflanzensubstanz durch die räuberische Blumenwanze *Anthrenorhinus nemorum*. *Entomophaga*, 24(4), pp 431–441.
- Lehman, H. (1932). Wanzen (Hemiptera-Heteroptera) als Obstbaumschädlinge. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, 42, pp 440–451.
- Lehrman, A., Boddum, T., Stenberg, J. A., Orians, C. M. & Björkman, C. (2013). Constitutive and herbivore-induced systemic volatiles differentially attract an omnivorous biocontrol agent to contrasting *Salix* clones. *AoB PLANTS*, 5(November 2012), pp plt005–plt005.
- Lehrman, A., Torp, M., Stenberg, J. A., Julkunen-Tiitto, R. & Björkman, C. (2012). Estimating direct resistance in willows against a major insect pest, *Phratora vulgatissima*, by comparing life history traits. *Entomologia Experimentalis et Applicata*, 144(1), pp 93–100.
- Lundgren, J. G., Hesler, L. S., Tilmon, K., Dashiell, K. & Scott, R. (2009). Direct effects of soybean varietal selection and *Aphis glycines*-resistant soybeans on natural enemies. *Arthropod-Plant Interactions*, 3(1), pp 9–16.
- Matassa, C. M. & Trussell, G. C. (2011). Landscape of fear influences the relative importance of consumptive and nonconsumptive predator effects. *Ecology*, 92(12), pp 2258–2266 Ecological Society of America.
- Matsumoto, K. (1990). Population dynamics of *Luehdorfia japonica* Leech (Lepidoptera : Papilionidae). II. Patterns of mortality in immatures in relation to egg cluster size. *Researches on Population Ecology*, 32, pp 173–188.
- Mayhew, P. J. (2001). Herbivore host choice and optimal bad motherhood. *Trends in ecology & evolution*, 16(4), pp 165–167.
- McCauley, S. J. & Rowe, L. (2011). The deadly effects of ‘nonlethal predators. *Ecology*, 92(11), pp 2043–2048 Eco Soc America.
- Miller, J. R. B., Ament, J. M. & Schmitz, O. J. (2014). Fear on the move: predator hunting mode predicts variation in prey mortality and plasticity in prey spatial response. *Journal of Animal Ecology*, 83(1), pp 214–222.
- Mitchell, R. (1975). The evolution of oviposition tactics in the bean weevil, *Callosobruchus maculatus* (F.). *Ecology*, 56(3), pp 696–702.
- Montserrat, M., Bas, C., Magalhães, S., Sabelis, M. W., De Roos, A. M. & Janssen, A. (2007). Predators induce egg retention in prey. *Oecologia*, 150(4), pp 699–705.
- Mulatu, B., Applebaum, S. W. & Coll, M. (2006). Effect of tomato leaf traits on the potato tuber moth and its predominant larval parasitoid: A mechanism for enemy-free space. *Biological Control*, 37(2), pp 231–236.
- Nelson, E. H. (2007). Predator avoidance behavior in the pea aphid: costs, frequency, and population consequences. *Oecologia*, 151(1), pp 22–32.
- Nelson, E., Matthews, C. & Rosenheim, J. (2004). Predators reduce prey population growth by inducing changes in prey behavior. *Ecology*, 85(7), pp 1853–1858.
- Peacock, L. & Herrick, S. (2000). Responses of the willow beetle *Phratora vulgatissima* to genetically and spatially diverse *Salix* spp. plantations. *Journal of Applied Ecology*, 37(5), pp 821–831.
- Peacock, L., Hunter, T., Turner, H. & Brain, P. (2002). Does host genotype diversity affect the distribution of insect and disease damage in willow cropping systems? *Journal of Applied Ecology*, 38(5), pp 1070–1081.
- Pilson, D. & Rausher, M. D. (1988). Clutch size adjustment by a swallowtail butterfly. *Nature*, 333(6171), pp 361–363 Nature Publishing Group.
- Preisser, E. L., Bolnick, D. I. & Benard, M. F. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, 86(2), pp 501–509 Eco Soc America.
- Roitberg, B. D., Robertson, I. C. & Tyerman, J. G. A. (1999). Vive la variance: a functional oviposition theory for insect herbivores. *Annual Review of Entomology*, (1978), pp 187–194.
- Rosenheim, J. A., Jepsen, S. J., Matthews, C. E., Smith, D. S. & Rosenheim, M. R. (2008).

- Time limitation, egg limitation, the cost of oviposition, and lifetime reproduction by an insect in nature. *The American Naturalist*, 172(4), pp 486–496.
- Schaller, A. (2008). *Induced plant resistance to herbivory*. Springer Verlag.
- Schmitz, O., Grabowski, J. & Peckarsky, B. (2008). From individuals to ecosystem function: toward an integration of evolutionary and ecosystem ecology. *Ecology*, 89(9), pp 2436–2445.
- Schmitz, O. J., Krivan, V. & Ovadia, O. (2004). Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecology Letters*, 7(2), pp 153–163.
- Siemens, D. & Johnson, C. (1992). Density-dependent egg parasitism as a determinant of clutch size in bruchid beetles (Coleoptera: Bruchidae). *Environmental Entomology*, 21(3), pp 610–619.
- Sigsgaard, L. (2010). Habitat and prey preferences of the two predatory bugs *Anthocoris nemorum* (L.) and *A. nemoralis* (Fabricius) (Anthocoridae: Hemiptera-Heteroptera). *Biological Control*, 53(1), pp 46–54 Elsevier Inc.
- Stenberg, J. A., Lehrman, A. & Björkman, C. (2010). Uncoupling direct and indirect plant defences: Novel opportunities for improving crop security in willow plantations. *Agriculture, Ecosystems and Environment*, 139(4), pp 528–533.
- Stenberg, J. a., Lehrman, A. & Björkman, C. (2011a). Host-plant genotype mediates supply and demand of animal food in an omnivorous insect. *Ecological Entomology*, 36(4), pp 442–449.
- Stenberg, J. A., Lehrman, A. & Björkman, C. (2011b). Plant defence: Feeding your bodyguards can be counter-productive. *Basic and Applied Ecology*, 12(7), pp 629–633 Elsevier GmbH.
- Stephan, J. G., Albertsson, J., Wang, L. & Porcel, M. (2016). Weeds within willow short-rotation coppices alter the arthropod community and improve biological control of the blue willow beetle. *BioControl*, 61(1), pp 103–114.
- Stephan, J. G., Stenberg, J. A. & Björkman, C. (2015). How far away is the next basket of eggs? Spatial memory and perceived cues shape aggregation patterns in a leaf beetle. *Ecology*, 96(4), pp 908–914.
- Subinprasert, S. & Svensson, B. W. (1988). Effects of predation on clutch size and egg dispersion in the codling moth *Laspeyresia pomonella*. *Ecological Entomology*, 13, pp 87–94.
- Tack, A. J. M., Ovaskainen, O., Pulkkinen, P. & Roslin, T. (2010). Spatial location dominates over host plant genotype in structuring an herbivore community. *Ecology*, 91(9), pp 2660–2672.
- Tammaru, T., Kaitaniemi, P. & Ruohomäki, K. (1996). Realized fecundity in *Epirrita autumnata* (Lepidoptera: Geometridae): Relation to body size and consequences to population dynamics. *Oikos*, 77(3), pp 407–416.
- Tatar, M. (1991). Clutch size in the swallowtail butterfly, *Battus philenor*: the role of host quality and egg load within and among seasonal flights in California. *Behavioral Ecology and Sociobiology*, 28(5), pp 337–344.
- Thaler, J. S., Contreras, H. & Davidowitz, G. (2014). Effects of predation risk and plant resistance on *Manduca sexta* caterpillar feeding behaviour and physiology. *Ecological Entomology*, 39(2), pp 210–216.
- Travers, S. E. & Sih, A. (1991). The influence of starvation and predators on the mating behavior of a semiaquatic insect. *Ecology*, 72(6), pp 2123–2136.
- Trussell, G. C., Ewanchuk, P. J. & Bertness, M. D. (2003). Trait-mediated effects in rocky intertidal food chains: Predator risk cues alter prey feeding rates. *Ecology*, 84(3), pp 629–640 ECOLOGICAL SOC AMER.
- Tschanz, B., Schmid, E. & Bacher, S. (2005). Host plant exposure determines larval vulnerability - do prey females know? *Functional Ecology*, 19(3), pp 391–395.
- Underwood, N. & Rausher, M. (2000). The effects of host-plant genotype on herbivore population dynamics. *Ecology*, 81(6), pp 1565–1576.
- Unsicker, S. B., Alexandra, F., Juliane, S., G??nter, K., Jeanine, L., Carsten, R., Claudia, S. & Weisser, W. W. (2010). Plant species richness in montane grasslands affects the fitness of a generalist grasshopper species. *Ecology*, 91(4), pp 1083–1091.
- Uzendoski, K., Maksymovitch, E. & Verrell, P. (1993). Do the Risks of Predation and Intermale Competition Affect Courtship Behavior in the Salamander *Desmognathus ochrophaeus*. *Behavioral Ecology and Sociobiology*, 32(6), pp 421–427.
- Vine, I. (1971). Risk of visual detection and pursuit by a predator and the selective advantage of flocking behaviour. *Journal of Theoretical Biology*, 30(2), pp 405–422.
- Wajnberg, E., Bernstein, C. & Van Alphen, J.

(2008). *Behavioral ecology of insect parasitoids: from theoretical approaches to field applications*. Blackwell Publishing.

Walzer, A. & Schausberger, P. (2009). Non-consumptive effects of predatory mites on thrips and its host plant. *Oikos*, 118(6), pp 934–940.

Wimp, G. M., Murphy, S. M., Finke, D. L., Huberty, A. F. & Denno, R. F. (2010). Increased primary production shifts the structure and composition of a terrestrial arthropod community. *Ecology*, 91(11), pp 3303–3311 Eco Soc America.

Young, A. & Moffett, M. (1979). Studies on the population biology of the tropical butterfly *Mechanitis isthmia* in Costa Rica. *American Midland Naturalist*, 101(2), pp 309–319.

Appendix

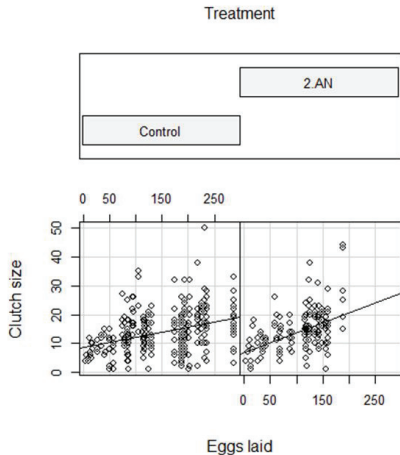


Figure S1: Clutch size in relation to number of eggs laid on a plant from the first experimental part (Control = only leaf beetles, AN = *Anthocoris nemorum*). In presence of AN the clutch size increases steeper with number of eggs laid on a plant. Lines indicate linear predictions.

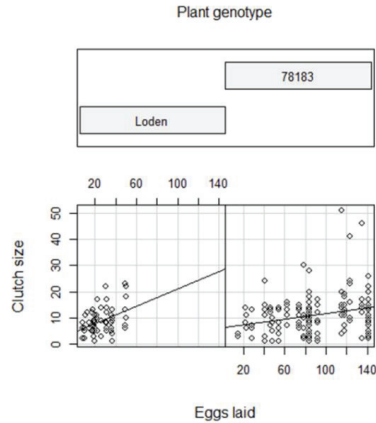


Figure S2: Clutch size in relation to number of eggs laid on a plant from the second experimental part (Control = only leaf beetles, AN = *Anthocoris nemorum*, OM = *Orthotylus marginalis*). On the plant genotype Loden the clutch size increases steeper with number of eggs laid on a plant. Lines indicate linear predictions.

Moving up and down the shoot: how ovipositing leaf beetles avoid habitat domains of different predators

Jörg G. Stephan^{1*}, Johan A. Stenberg², and Christer Björkman¹

¹ Department of Ecology, Unit of Forest Entomology, Swedish University of Agricultural Sciences, PO Box 7044, SE-75007 Uppsala, Sweden

² Department of Plant Protection Biology, Unit of Integrated Plant Protection, Swedish University of Agricultural Sciences, PO Box 102, SE-23053 Alnarp, Sweden

*Corresponding author e-mail: jorg.stephan@slu.se

Abstract

Ovipositing females need to consider the availability of food and the risk of predation when selecting ovipositing sites for their progeny. Predator presence during oviposition indicates higher predation risk and might induce the non-consumptive effect of avoiding high-risk oviposition sites. We hypothesized that the blue willow leaf beetle (*Phratora vulgatissima*) would choose larger leaves to ensure larval food provision and avoid the habitat domains of two important omnivorous predators on willow shoots of four different *Salix* varieties that are differently suitable to the herbivore but also the omnivores. We showed that among all possible leaf choices females more frequently chose large leaves for feeding and oviposition and fed more and laid more eggs. Females also preferentially fed in the shoot canopy and moved to the lower shoot part for oviposition. We characterized the habitat domains of the omnivorous predators that exhibit different hunting modes. The ‘run and eat’ predator *Anthocoris nemorum* foraged for eggs in the shoot canopy and its presence during beetle oviposition resulted in an amplified preference to oviposit farther down the shoot, although the strength of this response was also modulated by plant variety offering different sap quality to the omnivore. In contrast, independent of the plant variety, the ‘find and stay’ predator *Orthotylus marginalis* mainly consumed eggs in the lower shoot part and its presence during beetle oviposition did not lead to strong avoidance. If faced with both predators simultaneously the beetle’s lower shoot preference disappeared. Besides the known different hunting modes, the here described contrasting habitat domains provide another explanation to the weak interspecific interference between the two predator species. The plastic response of the beetles to different plant varieties and predators, however, indicates the involvement of predator avoidance during the evolution of oviposition site selection of this beetle species. We showed the applicability of the habitat domain concept in another system than grasslands and helped to understand species aggregation in a tritrophic context.

Keywords: fear, foraging behaviour, neutral interference, plant genotype, non-consumptive effect, tritrophic interaction, foraging mode

Introduction

In insect ecology, oviposition site choice is a key life history trait (Rosenheim *et al.*, 2008; Refsnider & Janzen, 2010) and important at the landscape scale (Meiners & Obermaier, 2004) as well as at the level of individual plants (Kessler & Baldwin, 2002; Silva & Furlong, 2012). Ovipositing females must balance conflicting demands because they need to lay their eggs in places that provide a sufficient food source for the larvae and where predation risk is low (Kessler & Baldwin, 2002). However, most prey species will face multiple predators, and the possible differences in danger that these predators present to the progeny is an additional factor that females need to consider when selecting an oviposition site. Differences in danger could originate from the predators foraging mode

(Huey & Pianka, 1981) or hunting modes and habitat domains (Miller *et al.*, 2014). For example, a predator that has a small overlap with the prey habitat domain might be less dangerous, or at least perceived as less dangerous, by the prey because the chances of encountering the predator are lower.

Predators not only consume the prey, but can also induce changes in the prey’s behaviour. Such non-consumptive effects can have far-reaching impacts on trophic interactions (Beckerman *et al.*, 1997; Trussell *et al.*, 2003) and ecosystem functions (Schmitz *et al.*, 2008; Matassa & Trussell, 2011), and they often equal or exceed the effects of direct consumption (Schmitz *et al.*, 2004; Preisser *et al.*, 2005). An example of a non-consumptive effect — and one which will be addressed here — is the anticipated predation on the progeny that can change the oviposition site selection by females of

the prey species (Vonesh & Blaustein, 2010; Lee *et al.*, 2014).

From the predator's point of view, sharing a prey with another predator can result in facilitation, interference, or neutral relationships depending on the similarities in hunting mode and habitat domain between the predators (Crowder *et al.*, 1997; Schmitz, 2007; Carey & Wahl, 2010). In general, variations in foraging behaviour can lead to the coexistence of related consumer species (Wilson *et al.*, 1999; Chase *et al.*, 2001). It is becoming more and more evident that many predators are omnivores (Rosenheim & Corbett, 2003) and it has been shown that lower plant quality due to induced resistance can increase the amount of feeding on the herbivorous food supply (Agrawal *et al.*, 1999) and that omnivorous insect predators may be more affected by plant quality than the herbivorous prey they consume (Eubanks & Denno, 1999). Omnivorous predators may also abandon discovered prey due to the requirement of a mixed diet (Mayntz *et al.*, 2005; Vasseur & Fox, 2011), but this abandonment can also differ based on how suitable the plant sap is. Therefore, we investigated whether different plant varieties have bottom up effects on the predator-prey interaction by using four very different and well investigated commercial willow varieties (*Salix viminalis*: 78183, 78021; *Salix dasyclados*: Gudrun, Loden) that differently affect the leaf beetle and its omnivorous predators (Stenberg *et al.*, 2010, 2011). Other important life history traits that could be affected by the presence of predators are oviposition rate and clutch size of the leaf beetle (Stephan *et al.* manuscript). However, here we will only focus on the locality of feeding and oviposition and how it is modulated by predator presence.

We showed previously that two important omnivorous predators of eggs and young larvae of the blue willow leaf beetle *Phratora vulgatissima* (Coleoptera: Chrysomelidae) show distinctly different hunting modes: *Anthocoris nemorum* (Heteroptera: Anthocoridae) exhibits a 'run and eat' foraging behaviour, while *Orthotylus marginalis* (Heteroptera: Miridae) is less mobile and can be characterized as a 'find and stay' predator (Björkman *et al.*, 2003). The different modes were used to explain how the predation rate on leaf beetle eggs and larvae is negatively affected by intraspecific interactions in the mobile predator *A. nemorum* but not in the less mobile predator *O. marginalis* (Björkman & Liman, 2005). The different hunting modes have also been used to explain the neutral interspecific interactions between the two predators observed both in the laboratory and in the field (Björkman & Liman, 2005). However, no study has investigated the habitat domains of these predators or the habitat domain of the shared leaf beetle prey although their overlap could also be

important to explain the predator-predator and predator-prey interaction on a plant individual. In general support for the concept of habitat domain only comes from grassland systems with planthoppers (Woodcock & Heard, 2011) and grasshoppers (Miller *et al.*, 2014). Here we will for the first time employ this concept for a woody plant species fed upon by leaf beetles. The system is also simple as willow shoots only provide a vertical, no horizontal domain.

Our first experiment aimed to quantify the observations that leaf beetles preferentially feed in the upper part of the shoot and moves to the lower part for oviposition leading to green leaves remaining only in the middle of the shoot in willow plantations (Mainsner, 1974). We hypothesized that the beetles should preferentially utilize large (resource rich) leaves for feeding and oviposition. Larger leaves should reduce intraspecific competition (Whitham, 1978) and improve the odds of survival because forcing the larvae to move to new sources of food after their initial food source is consumed could increase predation risk (Moreau & Björkman, 2012). Our second experiment sought to characterize the habitat domains of two of the main predators of the leaf beetle because it could help to understand if predation pressure could have been involved in the evolution of the vertical preference of the leaf beetle. We hypothesized that the two predator species have contrasting vertical preferences on the willow shoots for foraging on leaf beetle egg clutches. Our third experiment tested if the choice of oviposition sites by the leaf beetle has a plastic component with regard to the plant quality (here: variety) and the predator type. This experiment was performed on four *Salix* varieties on which we let leaf beetles oviposit in predator absence, presence of each predator (two individuals), and presence of both predators (one individual each, Table 1). We hypothesized that in the presence of single predator species the leaf beetle would avoid the habitat domain of that respective predator and if both predators are present we expect to find no preference for any position on the shoot. We further hypothesized that the vertical oviposition preference would be similar on different *Salix* varieties.

Materials and methods

Study system and general set up

Phratora vulgatissima has a wide distribution across Europe and Asia. The adults and larvae skeletonize willow (*Salix* spp.) leaves, and this species is an important herbivore on willow in Europe (Peacock & Herrick, 2000; Peacock *et al.*, 2002) that frequently reaches outbreak densities in natural willow stands and plantations (Björkman *et al.*, 2000; Dalin *et al.*, 2009).

Table 1: Overview of the three experiments with respective treatment and *Salix* variety combinations (*S. dasyclados*: Loden, Gudrun; *S. viminalis*: 78183, 78021) and the number of replications (individual plants). AN = *Anthrenus nemorum*; OM = *Orthotylus marginalis*; Control = only two leaf beetles

Exp.	Treatment	Variety	N
1	5 leaf beetles	78183	15
		78183	11
		78021	11
		Loden	10
		Gudrun	11
2	3 AN	78183	9
		78021	9
		Loden	9
		Gudrun	9
		78183	10
3	3 OM	78021	10
		Loden	10
		Gudrun	6
		78183	10
		78021	8
3	2 AN	Loden	8
		Gudrun	4
		78183	8
		Loden	7
		78183	8
	2 OM	Loden	7
		78183	8
	1 AN + 1 OM	Loden	7
		78183	7
	4 OM	Loden	8

Adults overwinter in reeds or under the bark of trees (Björkman & Eklund, 2006), emerge in April, feed for about two weeks, mate, and subsequently lay hundreds of eggs on the undersides of leaves in clutches of 1–50 eggs. The new larvae hatch after around two weeks, feed gregariously on leaves during the first and second instar and then feed solitarily during the third instar (Kendall *et al.*, 1996). This is followed by pupation in the soil. Adults emerge in August, and after a short period of feeding they find hibernation sites. Adults probably excrete pheromones that attract other individuals (Peacock *et al.*, 2001). After two weeks of egg laying, the oviposition rate is stable and is a good proxy for total egg production. Oviposition rate shows no apparent link to the survival of adults and, therefore, can be used as a good indicator of leaf beetle fitness (Lehrman *et al.*, 2012).

The common flower bug *A. nemorum* is an important bio-control agent in apple orchards (Sigsgaard, 2010) and can consume large numbers of *P. vulgatissima* eggs (Björkman *et al.*, 2003). *A. nemorum* and *O. marginalis* are among the most common natural enemies of eggs and larvae of *P. vulgatissima* in willow plantations (Björkman *et al.*, 2003), and *O. marginalis* appears to play a relevant role in *P.*

vulgatissima population dynamics (Björkman *et al.*, 2004). *A. nemorum* is mainly regarded as a predator, but it also feeds on shallowly located fluids from the green parts of host plants (Lauenstein, 1979). *O. marginalis* was observed to be mainly predacious (Lehman, 1932), while other observations supported the impression that it can survive on a minimal amount of animal food but that it has a preference for such food (Kullenberg, 1944).

The four *Salix* varieties selected for the experiments were chosen because they differ in chemical composition (Lehrman *et al.*, 2012) and have previously been tested for their suitability for both the leaf beetle and the omnivorous predator *A. nemorum*. The suitability of these varieties for the leaf beetle has been found to increase in the order Gudrun > Loden > 78021 > 78183 (Stenberg *et al.*, 2010), whereas the suitability of the varieties for *A. nemorum* in the absence of prey follows the reverse order. If additional prey is present, the most suitable of these varieties for *A. nemorum* is variety 78183 and the suitability's of the varieties Gudrun, Loden, and 78021 are similar (Stenberg *et al.*, 2011). With regard to oviposition behaviour of the leaf beetle we found that beetle individuals adjust their aggregation level to the suitability of the genotype and use spatial memory for their intra-plant clutch distribution differently well (Stephan *et al.*, 2015).

We grew *Salix* shoots from 20 cm winter cuttings that were placed in cylindrical transparent plastic cages (H= 70 cm, D= 30 cm) with a net on top during the experiments. We attempted to standardize the plants (height ~60 cm) by removing side shoots, dried leaves, and leaves at the top that were not fully expanded, and the plants were then randomly assigned to the treatments. Beetles were collected in the Uppsala area and reared in rearing cages on *S. viminalis* or *S. cinerea* (a suitable native host) in the greenhouse prior to the experiment. The individuals used in the experiments consisted of a mixture of field collected and next generation beetles from the rearing cages that were randomly distributed throughout the treatments. Clones of the different varieties were grown from winter cuttings in the greenhouse where all experiments were performed (23°C, RH 80, L18:D6).

Experiment 1: Vertical position of feeding and oviposition by the herbivore

Here we were interested in where on the shoot beetle females lay their eggs and where they preferentially feed. We aimed to have many feeding and oviposition incidences to relate them to the vertical position on the shoot and also to the area of a leaf. Therefore this experiment was performed with the host-plant variety 78183 that is highly accepted by the beetle

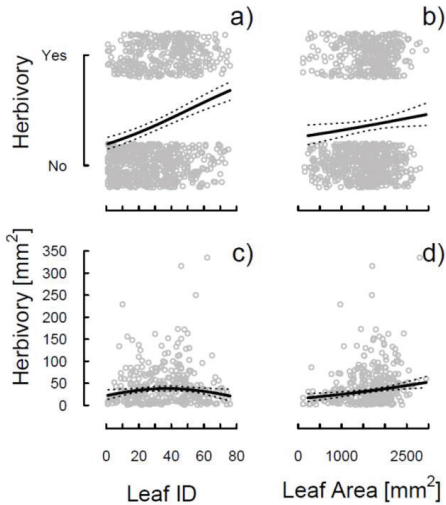


Figure 1: Herbivory of five leaf beetle (*Phratora vulgatissima*) females per *Salix viminalis* plant (variety: 78183) after nine days. Analysing all leaves on a shoot (Leaf ID = 1 is the lowest leaf on the shoot) showed that females more frequently chose leaves farther up the shoot for feeding (a) and larger leaves (b); despite the fact that large leaves are mostly in the middle of the shoot. Analysing only leaves chosen for feeding, females consumed similar amounts at all heights (c) but more from larger leaves (d). Circles show the incidence of herbivory (shifted to increase visibility) and absolute herbivory, and the lines indicate the model predictions with bootstrapped confidence limits.

(Stenberg *et al.*, 2011). To resemble the natural gregarious laying of some eggs followed by moving to another plant, we let each plant be visited by five ovipositing females during nine days. We started by releasing one female on each plant. Every morning the female was then removed for ~5 minutes (the time required for catch and release of the beetle) from the particular plant and released onto either the same or on the next plant among all plants. These changes were distributed over time and followed the pattern 10110101 (1 = release onto the next plant and 0 = release onto the same plant again). After nine days, we had 15 plants that had been visited by five different females each. This rather complicated experimental protocol was one among three treatments in a larger experiment where further explanations can be found (Stephan *et al.* 2015). After this procedure, we took photos of every leaf and which were analysed using ImageJ (NIH software, Bethesda, MD, USA). The numbers of eggs in these images were counted, and by drawing the missing leaf area in the skeletonized leaves we were able to measure the leaf area at the start of the experiment. Subtraction of the remaining leaf

area yielded the absolute herbivory on the leaf (see Appendix for detailed description).

Experiment 2: Vertical position of the herbivore egg consumption by the predators

Here we were interested in where on the shoot *A. nemorum* and *O. marginalis* preferentially forage on leaf beetle eggs. In order to describe where the predators are foraging we distributed leaf beetle egg clutches homogeneously along whole shoots on all four *Salix* varieties (Table 1); a situation that would not occur if eggs were laid naturally. Shoots of varieties 78183 and 78021 generally have more leaves than varieties Gudrun and Loden of the same height. Therefore, instead of leaf ID, each plant was divided into 13 equally sized parts (the uppermost position was part 13, and the lowest position was part 1) with several leaves in each part. In order to obtain experimental egg clutches for this experiment we

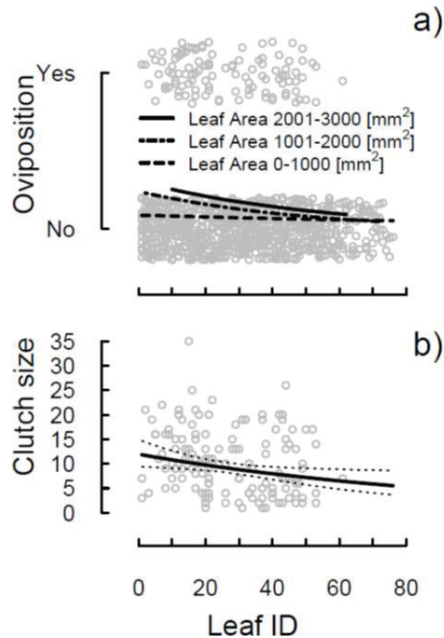


Figure 2: Oviposition of five leaf beetle (*Phratora vulgatissima*) females per *Salix viminalis* plant (variety: 78183) after nine days. Analysing all leaves on a shoot (Leaf ID = 1 is the lowest leaf) showed that females more frequently chose leaves further down the shoot (a), and the strength of these response increased with increasing leaf area. Analysing only the leaves chosen for oviposition, females laid larger clutches farther down the shoot (b). Circles show the incidence of oviposition (shifted to increase visibility) and eggs within clutch, and the lines indicate the model predictions with bootstrapped confidence limits.

removed leaves with egg clutches from the rearing cages. Clutches were manipulated to three size classes (5, 15, or 45 eggs per clutch) by carefully removing eggs under the microscope with a forceps, and these leaves were then attached with insect pins to leaves on the experimental plants. The attached leaves with the clutches dried out rapidly and did not provide food for the omnivorous predators and this method does not affect the eggs (K. Eklund, unpublished data). All 13 vertical positions of the experimental plants were randomly provided with one clutch while the total number of eggs on each plant and number of eggs in each size class were the same (one clutch of 45 eggs + three clutches of 15 eggs + nine clutches of 5 eggs = 135 eggs/plant = 13 clutches/plant). These different clutch sizes were chosen to represent the natural size distribution of ovipositing females, but will not be further analysed here. Due to the random vertical assignment clutch size is not interfering with investigating the position effect. The total numbers of eggs on each plant are typical of egg numbers observed during outbreak years. Three *A. nemorum* individuals or three *O. marginalis* individuals were then allowed to predate on the clutches for three days after which the number of empty egg shells and their locations were noted.

Experiment 3: Changes in vertical position of herbivore egg-laying due to predators

In this experiment, we investigated the effect of different *Salix* varieties and the presence of the predator *A. nemorum*, the predator *O. marginalis*, and the presence of both predators simultaneously on the egg-laying behaviour of the leaf beetles. All treatments were performed on equally treated and divided shoots as in the previous experiment. The first part of this experiment consisted of all four *Salix* varieties with either two ovipositing *P. vulgatissima* females caged on an individual plant for six days (Control) and one treatment with additionally two *A. nemorum* individuals (2 AN). Because we saw similar responses on both varieties of each *Salix* species we continued in the second part of the experiment only with the varieties 78183 and Loden (Table1). In this second part we added the following predators to individually caged plants with ovipositing females: two *A. nemorum* (2 AN), two *O. marginalis* (2 OM), one *A. nemorum* and one *O. marginalis* (1 AN + 1 OM), or four *O. marginalis* (4 OM). At the end of each part the clutch size, the number of eggs predated, and the position of each clutch were recorded and the proportions of surviving eggs per clutch were calculated.

Statistical analyses

The herbivory and oviposition data from experiment 1 were analysed with two different mixed models. First

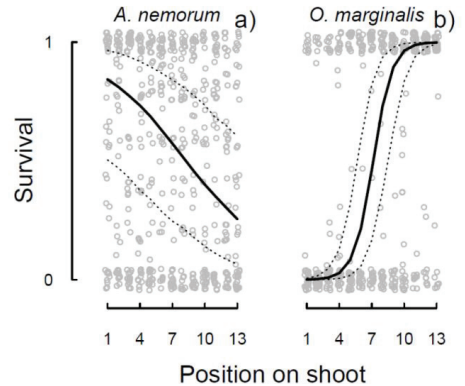


Figure 3: Survival of *Phratora vulgatissima* eggs within a clutch after being exposed to three *Anthocoris nemorum* (a) or three *Orthotylus marginalis* predators (b) in relation to vertical position of the clutch on the shoot (1 = lowest part along the shoot). The survival probability increased down the shoot if *A. nemorum* was released, whereas it increased up the shoot if *O. marginalis* was in the cage. Circles show the proportion survived eggs within clutch (shifted to increase visibility), and the lines indicate the model predictions with bootstrapped confidence limits.

we used all available leaves and models with a binomial distribution. Next the clutch size and absolute herbivory were analysed by using only the leaves that received herbivory/eggs with a model using a Poisson distribution (for clutch size) or a linear mixed model (for absolute herbivory). After visual inspection for normal distribution (Zuur *et al.*, 2010), the absolute herbivory was square root transformed. To account for the larger leaf area in the middle of the plants (Appendix: Fig. S1), a polynomial term for leaf identity was left in model M1 to M4 if significant differences between models were found. The data from experiments 2 and 3 were analysed with mixed models with a binomial/Poisson models, nested Gaussian random factors were included until the observational level to account for the hierarchical data structure and possible model overdispersion. The complete analysis was conducted using R (R Core Team, 2014). Further descriptions of the used R packages, the model selection and how the slopes were compared can be found in the Appendix.

Results

Experiment 1: Vertical position of feeding and oviposition by the herbivore

Because we included a polynomial term and a Leaf ID \times Leaf Area interaction that mostly were not significant or improved the model we were able to separate the effects of leaf area and vertical position of

Table 2: Analysis-of-deviance tables (Type III test) from linear/generalized linear mixed models investigating the feeding and oviposition behaviour of the leaf beetle *Phratora vulgatissima* (Exp. 1) and the predation behaviour of the omnivores *Anthrenus nemorum* and *Orthotylus marginalis* (Exp. 2). Leaf Area = leaf area of individual leaf; Leaf ID = identity of every leaf on the shoot. Predator = insect predator species; Position = vertical position of leaf assigned to 13 equally spaced parts on a plant; Variety = plant variety (*S. dasyclados*: Gudrun, Loden; *S. viminialis*: 78021, 78183). Non-significant terms (italicized) were removed stepwise from the final model starting from the bottom row. “/” means “nested within”; Obs = each observation

Model	Model Type	Random factor	Response Variable	Explanatory Variables	X ²	Df	AIC	R ² _{GLMM(m)}	R ² _{GLMM(c)}	p-value
Vertical position/leaf size preference for which leaf to choose for feeding on the plant variety 78183?										
M1	GLMM (Binomial)	Plant	Herbivory (Yes/No)	intercept	48.68	1	1132.81	0.09	0.11	<0.001
				Leaf Area	4.73	1	1132.81	0.09	0.11	0.02
				Leaf ID	46.78	1	1132.81	0.09	0.11	<0.001
				<i>Leaf Area × Leaf ID</i>	<i>0.87</i>	<i>1</i>	<i>1134.62</i>	<i>0.09</i>	<i>0.11</i>	<i>0.35</i>
Vertical position/leaf size preference for how much to feed on the plant variety 78183?										
M2	LMM	Plant	Square root of herbivory [mm ²]	intercept	17.50	1	1724.41	<i>0.07</i>	<i>0.07</i>	<0.001
				Leaf Area	11.47	1	1724.41	<i>0.07</i>	<i>0.07</i>	<0.001
				Leaf ID	3.87	1	1724.41	<i>0.07</i>	<i>0.07</i>	0.04
				(Leaf ID) ²	3.85	1	1724.41	<i>0.07</i>	<i>0.07</i>	0.04
				<i>Leaf Area × Leaf ID</i>	<i>0.26</i>	<i>1</i>	<i>1746.43</i>	<i>0.07</i>	<i>0.07</i>	<i>0.6</i>
Vertical position/leaf size preference for which leaf to choose for oviposition on the plant variety 78183?										
M3	GLMM (Binomial)	Plant	Oviposition (Yes/No)	intercept	55.80	1	<i>651.74</i>	<i>0.05</i>	<i>0.05</i>	<0.001
				Leaf Area	16.43	1	<i>651.74</i>	<i>0.05</i>	<i>0.05</i>	<0.001
				Leaf ID	0.56	1	<i>651.74</i>	<i>0.05</i>	<i>0.05</i>	0.45
				<i>Leaf Area × Leaf ID</i>	<i>14.95</i>	<i>1</i>	<i>651.74</i>	<i>0.05</i>	<i>0.05</i>	<0.001
Vertical position/leaf size preference for how many eggs to lay in a clutch on the plant variety 78183?										
M4	GLMM (Poisson)	Plant/Obs	Clutch size (eggs in clutch)	intercept	222.64	1	894.33	0.07	0.07	<0.001
				Leaf Area	1.70	1	894.33	0.07	0.07	0.19
				Leaf ID	5.83	1	894.33	0.07	0.07	0.01
				<i>Leaf Area × Leaf ID</i>	<i>1.42</i>	<i>1</i>	<i>894.91</i>	<i>0.08</i>	<i>0.08</i>	<i>0.23</i>
Vertical preference in predation by <i>A. nemorum</i> and <i>O. marginalis</i> on the plant varieties 78183, 78021, Loden and Gudrun?										
M5	GLMM (Binomial)	Plant/Obs	Survival (survived/predated)	intercept	4.75	1	3152.44	<i>0.16</i>	<i>0.93</i>	0.02
				Predator	53.16	1	3152.44	<i>0.16</i>	<i>0.93</i>	<0.001
				Position	11.39	1	3152.44	<i>0.16</i>	<i>0.93</i>	<0.001
				Predator × Position	128.06	1	3152.44	<i>0.16</i>	<i>0.93</i>	<0.001
				<i>Variety</i>	<i>3.71</i>	<i>3</i>	<i>3154.76</i>	<i>0.18</i>	<i>0.93</i>	<i>0.29</i>
				<i>Predator × Variety</i>	<i>0.86</i>	<i>3</i>	<i>3160.25</i>	<i>0.19</i>	<i>0.93</i>	<i>0.83</i>
				<i>Variety × Position</i>	<i>3.03</i>	<i>3</i>	<i>3163.10</i>	<i>0.19</i>	<i>0.93</i>	<i>0.38</i>
				<i>Predator × Variety × Position</i>	<i>0.34</i>	<i>3</i>	<i>3170.09</i>	<i>0.20</i>	<i>0.93</i>	<i>0.95</i>

the leaf. Leaf area was largest in the middle part of the shoot; therefore the beetles not only utilized the most common-sized leaves. Out of all the leaves, female leaf beetles more often chose the leaves farther up the shoot and those with a greater area for feeding (Table 2: M1; Fig. 1a/b). If only the chosen leaves were considered, feeding was still affected by the leaf position and beetles consumed more leaf area on larger leaves (Table 1: M2; Fig. 1c/d). Including all possible leaves in the model for oviposition resulted in a two-way interaction between Leaf area and Leaf ID (Table 1: M3). Although the larger leaves had a steeper slope between Leaf ID and incident of oviposition, the females generally preferred to oviposit in the lower shoot part (Fig. 2a). Having chosen these large leaves for oviposition, leaf area did not determine the clutch size, but larger clutches were laid in the lower shoot part (Table 1: M4; Fig. 2b). By using all three treatments and the relative height of each clutch on a shoot (because absolute height in form of Leaf ID was

not available for all treatments), we could also show that the vertical preference for oviposition was independent of indirectly perceived conspecific density (Appendix: Table S1, Fig. S2).

Experiment 2: Vertical position of the herbivore egg consumption by the predators

The survival probability of leaf beetle eggs in a clutch depended on the plant variety, and we found an interaction of predator species and the vertical position of the clutch on the shoot (Table 1: M5). This interaction can be explained by the preference of *A. nemorum* to consume eggs in the upper part of the shoot and *O. marginalis* to consume more eggs in the lower part (Fig. 3).

Experiment 3: Changes in vertical position of herbivore egg-laying due to predators

In the absence of predators, we could confirm the preference for ovipositing in the lower part of the shoot

for all four plant varieties (at a lower resolution with 13 positions) and a beetle density of two individuals (Table 3: M6; Fig. 4a-d). Comparing the slopes of all controls with the variety 78183 as reference indicated only a tendency ($p = 0.09$) of the slope of Loden being different while the remaining comparisons were not significant ($p > 0.1$). If the omnivorous predator *A. nemorum* was present during oviposition of the leaf beetle, the preference of ovipositing in the lower part of the shoot was amplified on both *S. viminalis* varieties (Fig. 4g, 4h) while on Gudrun we found a tendency. On the plant variety Loden, where the position effect was very strong already without predators present, we found no significant difference between slopes (Fig. 4f). The presence of *O. marginalis* did not prompt very strong changes in the vertical preference (Table 3: M7, Fig. 4) although there was a very strong tendency on Loden that the strong

position effect in predator absence was dampened (Fig. 5d). If both predators were in the cage the leaf beetles did respond by not showing any preference for any position anymore on the plant variety 78183 while we only found a tendency for the variety Loden (Fig. 5b, 5e). Increasing the predator density lead to mixed results with a stronger position effect on the plant variety 78183 (Fig. 5c) and a weaker on the plant variety Loden (Fig. 5f). For the egg survival in presence of two *A. nemorum* we found no interaction between plant variety and vertical position and, again, the egg survival decreased shoot upwards (Table 3: M8, Fig. 6a). For the remaining treatments in the second part of the experiment, survival was generally lower and we did not detect any differences between treatments but a slight increase in survival on 78183 on lower positions (Table 3: M9, Fig. 6b, 6c).

Table 3: Analysis-of-deviance tables (Type III test) from generalized linear mixed models investigating the oviposition behaviour of the leaf beetle *Phratora vulgatissima* and how it is altered by the presence of different predators (Exp. 3). Variety = plant variety; P-Treatment = always two leaf beetles with varying combination and number of predators; Position = vertical position of leaf assigned to 13 equally spaced parts on a plant. Non-significant terms (italicized) were removed stepwise from the final model starting from the bottom row. “/” means “nested within”; Obs = each observation

Model	Model Type	Random factor	Response Variable	Explanatory Variables	X ²	Df	AIC	R ² _{GLMM(m)}	R ² _{GLMM(c)}	p-value
Oviposition positions depend on predator treatments (Control/2 AN) on plant varieties 78183, 78021, Gudrun and Loden?										
M6	GLMM (Binomial)	Plant/ Obs	Oviposition (Yes/No)	intercept	25.03	1	1056.51	0.45	0.48	<0.001
				Variety	22.51	3	1056.51	0.45	0.48	<0.001
				P-Treatment	2.32	1	1056.51	0.45	0.48	0.12
				Position	35.33	1	1056.51	0.45	0.48	<0.001
				Variety × P-Treatment	8.68	3	1056.51	0.45	0.48	0.03
				Variety × Position	7.12	3	1056.51	0.45	0.48	0.06
				P-Treatment × Position	10.97	1	1056.51	0.45	0.48	<0.001
				Variety × P-Treatment × Position	8.00	3	1056.51	0.45	0.48	0.04
Oviposition positions depend on the predator treatments (Control ¹ /2 OM/1 AN + 1OM/4 OM) on plant varieties 78183 and Loden?										
M7	GLMM (Binomial)	Plant/ Obs	Oviposition (Yes/No)	intercept	17.70	1	1158.26	0.22	0.24	<0.001
				Variety	1.31	1	1158.26	0.22	0.24	0.25
				P-Treatment	12.22	3	1158.26	0.22	0.24	<0.01
				Position	10.25	1	1158.26	0.22	0.24	<0.01
				Variety × P-Treatment	6.12	3	1158.26	0.22	0.24	0.10
				Variety × Position	2.80	1	1158.26	0.22	0.24	0.09
				P-Treatment × Position	14.07	3	1158.26	0.22	0.24	<0.01
				Variety × P-Treatment × Position	11.46	3	1158.26	0.22	0.24	<0.01
Egg survival in clutches depend on position and predator treatment (2 AN) on plant varieties 78183, 78021, Gudrun and Loden ?										
M8	GLMM (Binomial)	Plant/ Obs	Survival (survived/ predated)	intercept	44.20	1	532.64	0.16	0.22	<0.001
				Position	22.92	3	532.64	0.16	0.22	<0.001
				Variety	3.87	1	532.64	0.16	0.22	<0.05
				<i>Variety × Position</i>	7.70	3	530.57	0.20	0.21	0.05
Egg survival in clutches depend on position and predator treatment (2 OM/1 AN + 1OM/4 OM) on plant varieties 78183 and Loden?										
M9	GLMM (Binomial)	Plant/ Obs	Survival (survived/ predated)	intercept	12.68	306.11	0.15	0.19	<0.001	
				Variety	2.58	306.11	0.15	0.19	0.10	
				P-Treatment	46.87	306.11	0.15	0.19	<0.001	
				Position	34.42	306.11	0.15	0.19	<0.001	
				Variety × P-Treatment	16.10	306.11	0.15	0.19	<0.001	
				Variety × Position	5.46	306.11	0.15	0.19	0.01	
				P-Treatment × Position	0.59	326.83	0.31	0.35	0.74	
				<i>Variety × P-Treatment × Position</i>	1.24	337.78	0.37	0.53	0.53	

Note: ¹ in order to compare the slopes of the treatments the Controls (from the first part of the experiment) were included

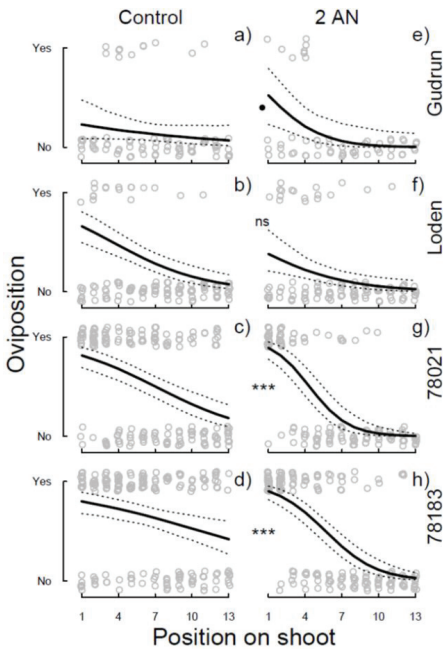


Figure 4: Oviposition of two *Phratra vulgatissima* females in relation to vertical position on the shoot (1 = lowest part along the shoot) with respect to different predator treatments (Control = only leaf beetles females, 2 AN = leaf beetles and two *Anthocoris nemorum* individuals) and plant variety (*Salix dasyclados*: Gudrun, Loden; *Salix viminalis*: 78021, 78183). Circles show the incidence of oviposition (shifted to increase visibility), and the lines show the model predictions with bootstrapped confidence limits (comparisons to slope of respective Control: *** = $p < 0.001$; ● = $p < 0.09$; ns = $p \geq 0.05$).

Discussion

The results of experiment 1 show that female leaf beetles more frequently initially select large leaves for feeding and feed more on larger leaves. Both behaviours make sense because the emerging larvae can save energy and reduce their risk of detection by predators if they do not have to move as often between leaves. The leaf beetle's preference for large leaves for oviposition became stronger farther down the shoot. This preference also makes sense because choosing large leaves for their progeny should decrease food competition among the larvae. Most previous feeding preference bioassays in the literature are somewhat artificial because they have used detached leaves or leaf discs thus imposing conditions that could affect the experimental results (Jones & Coleman, 1988; Dalin & Björkman, 2003). With our less artificial set-

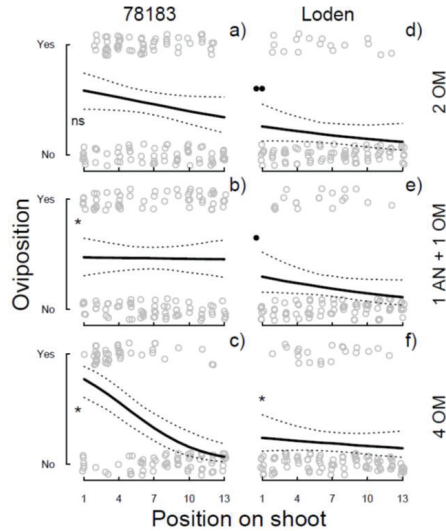


Figure 5: Oviposition of two *Phratra vulgatissima* females in relation to vertical position on the shoot (1 = lowest part along the shoot) with respect to plant variety (*Salix dasyclados*: Loden; *Salix viminalis*: 78183) and different predator treatments (Control = only leaf beetles (see Fig. 4b and 4d, respectively), AN = *Anthocoris nemorum*, OM = *Orthotylus marginalis*). Circles show the incidence of oviposition (shifted to increase visibility), and the lines show the model prediction with bootstrapped confidence limits (comparisons to slope of respective Control: * = $p < 0.05$; ● = $p < 0.09$; ns = $p \geq 0.05$).

up, we were able to demonstrate that larger leaf area is favoured for oviposition but only if all possible choices on the shoot are considered. This result would have been hidden if we had investigated only the relationship between clutch size and the area of leaves on which oviposition occurred.

The results also showed that leaf beetle females preferred to feed on the upper part of the shoot while oviposition occurred mainly on the lower part. Despite the fact that leaves are smaller on the top and bottom of the plant it was clear that leaf area and vertical position of the leaf on the plant are important determinants for beetle foraging and oviposition.

Higher nutritional value (Coley, 1980) could be the reason for adults grazing on young canopy leaves and for three of the four here used willow varieties it has been shown that leaf nitrogen concentration increases along the shoot with highest values in the canopy (Weih & Rönnberg-Wästjüng, 2007). It is therefore unexpected that females disperse to the lower shoot for oviposition and lay larger clutches farther down the shoot and it calls for another explanation.

Based on the assumption that predation risk influences the oviposition behaviour, the second

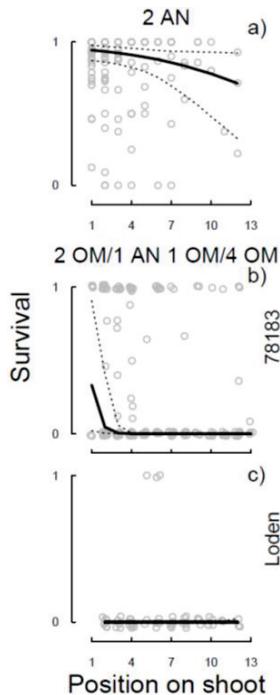


Figure 6: Egg survival within leaf beetle (*Phratora vulgatissima*) clutches in relation to vertical position on the shoot (1 = lowest part along the shoot) and predator treatments (AN = *A. nemorum*, OM = *O. marginalis*). There were no differences among the four varieties for the treatment with two *A. nemorum* (a) and no treatment-variety interaction for the second part of the experiment (b and c). Circles show the proportion survived eggs within a clutch (shifted to increase visibility), and the lines show the model prediction with bootstrapped confidence limits.

objective of this study was to investigate two important predators in the system. We found that both omnivorous predators exhibit contrasting preferred hunting areas at the scale of individual plants. *A. nemorum* consumed eggs mainly in the upper part of the plant, whereas *O. marginalis* foraged preferentially in the lower part. The observation that none of the four plant varieties interfered with the contrasting habitat domains of the two predators further strengthens our interpretation that these are general attributes. Also, plant sap quality does not change along the shoot (Siebrecht *et al.*, 2003) and is not the reason for any vertical preferences. The predators appear, therefore, to have different habitat domains on young willow shoots which is the first indication of the applicability of habitat domain to other systems than grasslands. We believe that the different habitat domains of the predators is another important factor contributing to the neutral relationship between the two predator

species, which previously has been attributed solely to their different hunting modes (Björkman & Liman, 2005). Theoretically, the coexistence of predators with non-overlapping narrow domains and different hunting modes and a prey that has a narrow domain (here the lower shoot part) is not possible (Schmitz, 2007). The fact that both predators are omnivores and that *P. vulgatissima* is certainly not the only prey they consume is probably the reason for the mismatch with this theory and the reason why both predators can coexist in willow plantations (Björkman *et al.*, 2004). The reasons of the different hunting domains remain unexplored and still have to be confirmed in the field. We suspect that the fact that *A. nemorum* (overwintering as adult) feeds on flower nectar (Sigsgaard & Kollmann, 2007) and that *O. marginalis* hatches after willow flowering (overwinters as eggs) and mainly feeds on sap are relevant for the different domains. Also the very active *A. nemorum* might pass the lower shoot part occupied by *O. marginalis* and is forced to forage in an area with fewer beetle eggs. This would be in line with our impression that *O. marginalis* is more territorial than *A. nemorum* because it lays its eggs in the lower part of the shoot and actively defends them. The less dominant species often avoid the dominant species (Binz *et al.*, 2014) and *A. nemorum* may avoid time and energy-consuming confrontations. This confrontation avoidance seems plausible because the gregariousness and egg clustering behaviour of the leaf beetle leads to high egg abundances in certain areas and ensures sufficient food availability for the predators on the respective shoots.

The final objective of the study was to investigate if the beetle's habitat domain is apparent on different *Salix* varieties, if it is modulated by the contrasting habitat domains of the predators, and what would happen if both predators are present simultaneously. Although on a lower resolution (13 positions instead of leaf ID as in experiment 1) we could confirm the beetle females preferentially oviposit in the lower shoot part on all four varieties. In line with our hypotheses we could show that ovipositing beetles show a plastic response to the presence of the predators. However the plasticity was differently strong among predator treatments and plant varieties. The presence of *A. nemorum* amplified the behaviour of laying more eggs in the lower plant part, at least on three of the four plant varieties. The presence of *O. marginalis*, however, triggered some but no strong responses in the leaf beetles. The weaker response to *O. marginalis* could be a result of the previously mentioned shorter temporary overlap early in the spring, facilitated by the fact that the time to first reproduction and the survival of the herbivore eggs laid early in the season are generally believed to be very important (Parry *et al.*, 1998). Also the less mobile *O. marginalis* may be less

frequently encountered by the beetles than *A. nemorum* with its 'run and eat' behaviour making the shoot canopy appearing more dangerous. The fact that species in the family Anthicoridae produce alarm substances (Evans, 1976) that, at least in theory, could be used by the leaf beetle as a kairomone could support this interpretation. The situation with both predators present simultaneously resulted in no preference for any position and a tendency for a weakened preference for the lower shoot part. It validates that the females can perceive both predators and try to avoid their habitat domains. Because the predators are not harmful to the adult beetles, we can also infer that females anticipate future predation on their eggs/larvae. In the last treatment we increased *O. marginalis* density to four individuals. We expected the leaf beetles' preference for the lower shoot part to be even more weakened. We could confirm this on the variety Loden, but not on 78183 where we observed the opposite. This interaction between predator density and plant variety is puzzling and will be investigated further.

In contrast to experiment 2, where we pinned egg clutches on otherwise undamaged willow plants to describe the predator habitat domains, the plants in experiment 3 experienced natural oviposition meaning that herbivory, faeces, pheromones, and induced plant volatiles (Peacock *et al.*, 2001; Fernandez & Hilker, 2007) were included in the set up. Here the predators encountered the adult beetles and the habitat domain of the prey was overlaid by the domains of the predators. Regardless of the plant variety and despite the avoidance by the herbivore of the shoot canopy we could still confirm that beetle egg survival in clutches was lower in the canopy if *A. nemorum* was present. In contrast, survival was very low in the remaining predator treatments and we could only detect a slightly higher survival at the lowest positions on the variety 78183. The generally lower survival may be attributed to the fact that we had to use second to fourth instar *O. marginalis* in this second part of the experiment because we had to wait for the new generation of *A. nemorum*. Nevertheless, we still generally believe that the plastic predator avoidance behaviour would increase leaf beetle egg survival.

Several reasons might have caused the evolved behaviour of laying eggs in the lower shoot part. First, securing food provision by not laying eggs where leaf beetle adults have skeletonized the leaves may be important. Although past evolution cannot be excluded (Connell, 1980), we don't think that's likely. Our findings support this view as females showed the movement despite adult herbivory being very low in experiment 1 and 3. Additionally, neither indirectly perceived conspecific density (Appendix) nor different egg numbers on the different *Salix* varieties in

experiment 3 affected the movement. Second, leaf beetle males engage strongly in their mating behaviour (three individuals on top of each other in crowded cages are not uncommon) and mated females may try to avoid disturbance during oviposition. However, such disturbance may not occur naturally as we never observed three males on top of each other in the field. Third, defence traits could change along the shoot. Leaf trichome density on new leaves after the shoot experienced beetle attack increases, however this increase does not change larvae weight gain but may affect the second generation of beetles feeding on the same plants (but not reproduce in that season) (Dalin & Björkman, 2003; Dalin *et al.*, 2004). That we found the vertical preference on all four plant varieties that differ chemically (Lehrman *et al.*, 2012) also points to plant suitability being not relevant for the movement. Lastly, based on unpublished field data of two experiments investigating UV radiation and microclimate (standardized climatic conditions using fans) did not show any indications of affecting egg survival and are unlikely driver of this movement. Conversely, avoidance of a superior resource patch by herbivores due to predation threat is a known phenomenon (Ballabeni *et al.*, 2001; Meiners *et al.*, 2005), and we believe this is one important driving force for *P. vulgatissima* females to have evolved the behaviour of moving to the lower part of the shoot for oviposition. However, many predators and parasitoids can be found on willow shoots (Stephan *et al.*, 2016) and we do not believe this vertical preference has evolved due to the predation pressure by the investigated predators that are generalists which makes coevolution unlikely. Instead we do see both predators as the representatives for the variety of hunting modes and habitat domains and see the plastic response of the leaf beetle as evidence that predator avoidance could have been implicated in the evolution of the observed vertical preference.

In conclusion, the facts that *P. vulgatissima* females feed in the shoot canopy but oviposit in the lower part of the shoot, together with the unlikelihood of other driving factors, makes us suspect that avoiding predation is important in the oviposition behaviour of this beetle. The finding that two of the most important predators have contrasting hunting domains on *Salix* shoots is likely to contribute to their neutral interspecific relationship, in addition to their different hunting modes. Our results therefore contribute to the understanding of predator-predator interaction and how prey behaviour and predator behaviour interact. Knowledge about this tritrophic interaction will help to elucidate species aggregations.

Acknowledgements

We thank Caroline Jöngren and Staffan Matzén for their assistance during the experiment 1 and Mikael Andersson Franko for inspiring discussions about the statistical analysis. This study was funded by the Swedish Energy Agency (Energimyndigheten) and the Swedish Research Agency Formas.

References

- Agrawal, A. a., Kobayashi, C. & Thaler, J. S. (1999). Influence of prey availability and induced host-plant resistance on omnivory by western flower thrips. *Ecology*, 80(2), pp 518–523.
- Ballabeni, P., Włodarczyk, M. & Rahier, M. (2001). Does enemy-free space for eggs contribute to a leaf beetle's oviposition preference for a nutritionally inferior host plant? *Functional Ecology*, 15(3), pp 318–324.
- Beckerman, A. P., Uriarte, M. & Schmitz, O. J. (1997). Experimental evidence for a behavior-mediated trophic cascade in a terrestrial food chain. *Proceedings of the National Academy of Sciences of the United States of America*, 94(20), pp 10735–10738 The National Academy of Sciences of the USA.
- Binz, H., Foitzik, S., Staab, F. & Menzel, F. (2014). The chemistry of competition: exploitation of heterospecific cues depends on the dominance rank in the community. *Animal Behaviour*, 94, pp 45–53 Elsevier Ltd.
- Björkman, C., Bengtsson, B. & Häggström, H. (2000). Localized outbreak of a willow leaf beetle: plant vigor or natural enemies? *Population Ecology*, 42, pp 91–96.
- Björkman, C., Bommarco, R., Eklund, K. & Höglund, S. (2004). Harvesting disrupts biological control of herbivores in a short-rotation coppice system. *Ecological Applications*, 14(6), pp 1624–1633 Eco Soc America.
- Björkman, C., Dalin, P. & Eklund, K. (2003). Generalist natural enemies of a willow leaf beetle (*Phratora vulgatissima*): abundance and feeding habits. *Journal of Insect Behavior*, 16(6), pp 747–764.
- Björkman, C. & Eklund, K. (2006). Factors affecting willow leaf beetles (*Phratora vulgatissima*) when selecting overwintering sites. *Agricultural and Forest Entomology*, 8(2), pp 97–101.
- Björkman, C. & Liman, A.-S. (2005). Foraging behaviour influences the outcome of predator-predator interactions. *Ecological Entomology*, 30(2), pp 164–169.
- Carey, M. P. & Wahl, D. H. (2010). Interactions of multiple predators with different foraging modes in an aquatic food web. *Oecologia*, 162(2), pp 443–52.
- Chase, J. M., Wilson, W. G. & Richards, S. a. (2001). Foraging trade-offs and resource patchiness: theory and experiments with a freshwater snail community. *Ecology Letters*, 4(4), pp 304–312.
- Coley, P. D. (1980). Effects of leaf age and plant life history patterns on herbivory. *Nature*, 284(5756), pp 545–546.
- Connell, J. (1980). Diversity and the coevolution of competitors, or the ghost of competition past. *Oikos*, 35(2), pp 131–138.
- Crowder, L., Squires, D. & Rice, J. (1997). Nonadditive effects of terrestrial and aquatic predators on juvenile estuarine fish. *Ecology*, 78(6), pp 1796–1804.
- Dalin, P. & Björkman, C. (2003). Adult beetle grazing induces willow trichome defence against subsequent larval feeding. *Oecologia*, 134(1), pp 112–8.
- Dalin, P., Björkman, C. & Eklund, K. (2004). Leaf beetle grazing does not induce willow trichome defence in the coppicing willow *Salix viminalis*. *Agricultural and Forest Entomology*, 6(2), pp 105–109.
- Dalin, P., Kindvall, O. & Björkman, C. (2009). Reduced population control of an insect pest in managed willow monocultures. *PloS one*, 4(5), p e5487.
- Eubanks, M. D. & Denno, R. F. (1999). The ecological consequences of variation in plants and prey for an omnivorous insect. *Ecology*, 80(4), pp 1253–1266.
- Evans, H. F. (1976). Mutual interference between predatory anthocorids. *Ecological Entomology*, 1(4), pp 283–286.
- Fernandez, P. & Hilker, M. (2007). Host plant location by Chrysomelidae. *Basic and Applied Ecology*, 8(2), pp 97–116.
- Huey, R. B. & Pianka, E. R. (1981). Ecological consequences of foraging mode. *Ecology*, 62(4), pp 991–999.
- Jones, C. & Coleman, J. (1988). Leaf disc size and insect feeding preference: implications for assays and studies on induction of plant defense. *Entomologia Experimentalis et Applicata*, 47(2), pp 167–172.
- Kendall, D., Wiltshire, C. & Butcher, M. (1996). *Phenology and population dynamics of willow beetles (Coleoptera; Chrysomelidae) in short-rotation coppiced willows at Long Ashton. ETSU (DTI) Biofuels Study B/M4/00487/14/REP. IACR-Long Ashton*

Research Station.

- Kessler, A. & Baldwin, I. (2002). *Manduca quinquemaculata's optimization of intra-plant oviposition to predation, food quality, and thermal constraints. Ecology*, 83(8), p 2346.
- Kullenberg, B. (1944). Studien über die Biologie der Capsiden. *Zoologiska Bidrag från Uppsala*, 23, pp 240–243 Uppsala: Almqvist & Wiksellis Boktryeri AB.
- Lauenstein, G. (1979). Zur Aufnahme von Pflanzensubstanz durch die räuberische Blumenwanze *Anthocoris nemorum*. *Entomophaga*, 24(4), pp 431–441.
- Lee, D. H., Nyrop, J. P. & Sanderson, J. P. (2014). Non-consumptive effects of the predatory beetle *Delphastus catalinae* (Coleoptera: Coccinellidae) on habitat use patterns of adult whitefly *Bemisia argentifolii* (Hemiptera: Aleyrodidae). *Applied Entomology and Zoology*, 49(4), pp 599–606.
- Lehman, H. (1932). Wanzen (Hemiptera-Heteroptera) als Obstbaumschädlinge. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, 42, pp 440–451.
- Lehman, A., Torp, M., Stenberg, J. A., Julkunen-Tiitto, R. & Björkman, C. (2012). Estimating direct resistance in willows against a major insect pest, *Phratora vulgatissima*, by comparing life history traits. *Entomologia Experimentalis et Applicata*, 144(1), pp 93–100.
- Maisner, N. (1974). Chrysomelidae, Blattkäfer. In: Schwenke, W. (Ed) *Die Forstschädlinge Europas*. 2. ed, pp 202–236. Hamburg, Berlin: Verlag Paul Parey.
- Matassa, C. M. & Trussell, G. C. (2011). Landscape of fear influences the relative importance of consumptive and nonconsumptive predator effects. *Ecology*, 92(12), pp 2258–2266 Ecological Society of America.
- Mayntz, D., Raubenheimer, D., Salomon, M., Toft, S. & Simpson, S. J. (2005). Nutrient-specific foraging in invertebrate predators. *Science*, 307(5706), pp 111–113.
- Meiners, T., Hacker, N. K., Anderson, P. & Hilker, M. (2005). Response of the elm leaf beetle to host plants induced by oviposition and feeding: the infestation rate matters. *Entomologia Experimentalis et Applicata*, 115(1), pp 171–177.
- Meiners, T. & Obermaier, E. (2004). Hide and seek on two spatial scales—vegetation structure effects herbivore oviposition and egg parasitism. *Basic and Applied Ecology*, 5(1), pp 87–94.
- Miller, J. R. B., Ament, J. M. & Schmitz, O. J. (2014). Fear on the move: predator hunting mode predicts variation in prey mortality and plasticity in prey spatial response. *Journal of Animal Ecology*, 83(1), pp 214–222.
- Moreau, G. & Björkman, C. (2012). Nonadditive interactions between trophic levels bias the appraisal of the strength of mortality factors. *Population Ecology*, 54(1), pp 125–133.
- Parry, D., Spence, J. & Volney, W. (1998). Budbreak phenology and natural enemies mediate survival of first-instar forest tent caterpillar (Lepidoptera: Lasiocampidae). *Environmental Entomology*, 27(6), pp 1368–1374.
- Peacock, L. & Herrick, S. (2000). Responses of the willow beetle *Phratora vulgatissima* to genetically and spatially diverse *Salix* spp. plantations. *Journal of Applied Ecology*, 37(5), pp 821–831.
- Peacock, L., Hunter, T., Turner, H. & Brain, P. (2002). Does host genotype diversity affect the distribution of insect and disease damage in willow cropping systems? *Journal of Applied Ecology*, 38(5), pp 1070–1081.
- Peacock, L., Lewis, M. & Herrick, S. (2001). Factors influencing the aggregative response of the blue willow beetle, *Phratora vulgatissima*. *Entomologia Experimentalis et Applicata*, 98(2), pp 195–201.
- Preisser, E. L., Bolnick, D. I. & Benard, M. F. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, 86(2), pp 501–509 Eco Soc America.
- R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Refsnider, J. M. & Janzen, F. J. (2010). Putting eggs in one basket: Ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annual Review of Ecology, Evolution, and Systematics*, 41(1), pp 39–57.
- Rosenheim, J. A. & Corbett, A. (2003). Omnivory and the indeterminacy of predator function: Can a knowledge of foraging behavior help? *Ecology*, 84(10), pp 2538–2548.
- Rosenheim, J. A., Jepsen, S. J., Matthews, C. E., Smith, D. S. & Rosenheim, M. R. (2008). Time limitation, egg limitation, the cost of oviposition, and lifetime reproduction by an insect in nature. *The American Naturalist*, 172(4), pp 486–496.
- Schmitz, O. (2007). Predator diversity and trophic interactions. *Ecology*, 88(10), pp 2415–2426.
- Schmitz, O., Grabowski, J. & Peckarsky, B. (2008).

- From individuals to ecosystem function: toward an integration of evolutionary and ecosystem ecology. *Ecology*, 89(9), pp 2436–2445.
- Schmitz, O. J., Krivan, V. & Ovadia, O. (2004). Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecology Letters*, 7(2), pp 153–163.
- Siebrecht, S., Herdel, K., Schurr, U. & Tischner, R. (2003). Nutrient translocation in the xylem of poplar? diurnal variations and spatial distribution along the shoot axis. *Planta*, 217(5), pp 783–793.
- Sigsgaard, L. (2010). Habitat and prey preferences of the two predatory bugs *Anthocoris nemorum* (L.) and *A. nemoralis* (Fabricius) (Anthocoridae: Hemiptera-Heteroptera). *Biological Control*, 53(1), pp 46–54 Elsevier Inc.
- Sigsgaard, L. & Kollmann, J. (2007). Beneficial effects of hedgerow plants for insect predators in adjacent orchards—the value of pollen and nectar to *Anthocoris nemorum* (L.). *IOBC WPRS BULLETIN*, 30(4), pp 4–6.
- Silva, R. & Furlong, M. J. (2012). Diamondback moth oviposition: effects of host plant and herbivory. *Entomologia Experimentalis et Applicata*, 143(3), pp 218–230.
- Stenberg, J. A., Lehrman, A. & Björkman, C. (2010). Uncoupling direct and indirect plant defences: Novel opportunities for improving crop security in willow plantations. *Agriculture, Ecosystems and Environment*, 139(4), pp 528–533.
- Stenberg, J. a., Lehrman, A. & Björkman, C. (2011). Host-plant genotype mediates supply and demand of animal food in an omnivorous insect. *Ecological Entomology*, 36(4), pp 442–449.
- Stephan, J. G., Albertsson, J., Wang, L. & Porcel, M. (2016). Weeds within willow short-rotation coppices alter the arthropod community and improve biological control of the blue willow beetle. *BioControl*, 61(1), pp 103–114.
- Stephan, J. G., Stenberg, J. A. & Björkman, C. (2015). How far away is the next basket of eggs? Spatial memory and perceived cues shape aggregation patterns in a leaf beetle. *Ecology*, 96(4), pp 908–914.
- Trussell, G. C., Ewanchuk, P. J. & Bertness, M. D. (2003). Trait-mediated effects in rocky intertidal food chains: Predator risk cues alter prey feeding rates. *Ecology*, 84(3), pp 629–640 ECOLOGICAL SOC AMER.
- Vasseur, D. a & Fox, J. W. (2011). Adaptive dynamics of competition for nutritionally complementary resources: character convergence, displacement, and parallelism. *The American Naturalist*, 178(4), pp 501–514.
- Vonesh, J. & Blaustein, L. (2010). Predator-induced shifts in mosquito oviposition site selection: a meta-analysis and implications for vector control. *Israel Journal of Ecology & Evolution*, 56(3-4), pp 263–279.
- Weih, M. & Rönnerberg-Wästjung, A.-C. (2007). Shoot biomass growth is related to the vertical leaf nitrogen gradient in *Salix* canopies. *Tree physiology*, 27(11), pp 1551–1559.
- Whitham, T. (1978). Habitat selection by *Pemphigus* aphids in response to response limitation and competition. *Ecology*, 59(6), pp 1164–1176.
- Wilson, W., Osenberg, C., Schmitt, R. & Nisbet, R. (1999). Complementary foraging behaviors allow coexistence of two consumers. *Ecology*, 80(7), pp 2358–2372.
- Woodcock, B. A. & Heard, M. S. (2011). Disentangling the effects of predator hunting mode and habitat domain on the top-down control of insect herbivores. *Journal of Animal Ecology*, 80(2), pp 495–503.
- Zuur, A., Ieno, E. & Elphick, C. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, 1(1), pp 3–14.

Appendix

Picture analysis with ImageJ

Pictures of leaves on the shoots were taken with a Canon Digital IXUS 60. Behind each leaf we were holding a white sheet of paper that was equipped with a piece of 50 mm long red tape. Pictures were analyzed using ImageJ (Schneider *et al.*, 2012). For each picture, we set the scale by using the red tape as the internal standard (draw line on 50 mm stripe → *Analyze* → *Set Scale*). Each leaf was roughly surrounded with the Polygon tool followed by removal of the outside (*Edit* → *Clear outside*), converted to grayscale (*Image* → *Type* → *8-bit*), and made binary (*Process* → *Binary* → *Make Binary*) leaving the leaf in black on a white background. After measuring (*Set Measurements* within the Result window to *Area* and *Limit to threshold*), individual leaf areas were acquired. All commands after the selection were pooled into a macro and executed with a shortcut.

Statistical analysis with R:

We used the functions *lmer* and *glmer* from the package *lme4* (Bates *et al.* 2012) for the linear mixed

model (LMM) and the generalized linear mixed models (GLMM). The conditional and marginal pseudo R^2 values were calculated with the function *r.squaredGLMM* from the package *MuMIn* (Bartoń 2014). The type III analysis-of-variance tables were acquired with the function *Anova* from the package *car* (Fox and Weisberg 2011). Model predictions were obtained with the function *ezPredict* from the package *ez* (Lawrence and Lawrence 2013). To increase visibility the gained predictions and confidence limits of the Poisson and binomial models were transformed to linear predictions (while the data are presented in original form).

Model selection

We used the functions *lmer* and *glmer* from the package *lme4* (Bates *et al.*, 2012) for the linear mixed model (LMM) and the generalized linear mixed models (GLMM). The conditional and marginal pseudo R^2 values were calculated with the function *r.squaredGLMM* from the package *MuMIn* (Bartoń, 2014). The type III analysis-of-variance tables were acquired with the function *Anova* from the package *car* (Fox & Weisberg, 2011). Model predictions were obtained with the function *ezPredict* from the package *ez* (Lawrence & Lawrence, 2013). To increase visibility the gained predictions and confidence limits of the Poisson and binomial models were transformed to linear predictions (while the data are presented in original form).

References

- Bartoń, K. (2014). MuMIn: Multi-model inference. R package version 1.10.0. Retrieved May 14, 2014, from <http://cran.r-project.org/package=MuMIn>.
- Bates, D., Maechler, M. & Bolker, B. (2012). lme4: Linear mixed-effects models using Eigen and Eigen. Comprehensive R Archive Network.
- Fox, J. & Weisberg, S. (2011). An {R} Companion to Applied Regression. (Fox, J., Ed) Sage Publications Sage Publications. ISBN 141297514X.
- Lawrence, A. M. A. & Lawrence, M. M. A. (2013). Package 'ez'.
- Nakagawa, S. & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. (O'Hara, R. B., Ed) *Methods in Ecology and Evolution*, 4(2), pp 133–142.
- Schneider, C., Rasband, W. & Eliceiri, K. W. (2012). NIH image to imageJ: 25 years of image analysis. *Nature methods*, 9, pp 671–675.

Leaf area along the shoot

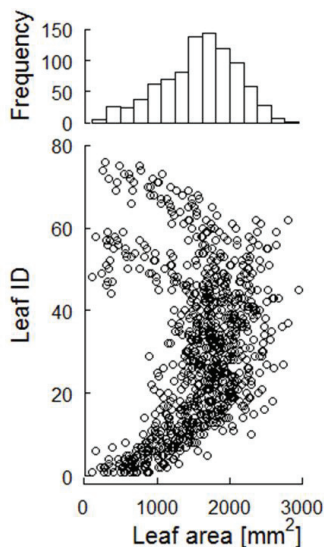


Figure S1: Leaf area of individual leaves of all *S. viminalis* plants depending on absolute position (Leaf ID = 1 is the lowest leaf) on the shoot. The largest leaf area can be found in the middle of the shoot and the abundance of leaves with each leaf area follows a normal distribution (mean, standard deviation, min, max of: plant height (633, 62, 530, 730); leaf number on each plant (60, 11, 38, 76); herbivory in mm^2 (16, 34, 0, 335); leaf area in mm^2 (1550, 546, 114, 2947)).

Indirectly perceived conspecific density and vertical oviposition preference

Experiment 1 was part of a larger experiment that investigated the spatial memory during oviposition and the aggregation on a plant depending on the indirectly perceived conspecific density (Paper I). The treatment utilized here (*mixed host*) for investigating the leaf area and vertical position effect had two other accompanying treatments (T): *same host* where we released the female repeatedly on the same plant and *new hosts* where we release the female exclusively on a new plant. For these two treatments, we did not measure the leaf areas or note the leaf ID the clutch was deposited on. However, the relative height (P.rel) of a clutch (clutch height above the ground/plant height) was available for all three treatments. For the *S. viminalis* genotype 78183, we tested if these different indirectly perceived conspecific densities affect the vertical oviposition preference. Regardless of the indirectly perceived conspecific density (Table S1), *P. vulgatissima* females showed a preference to oviposit in the lower parts of the shoot (Fig. S2).

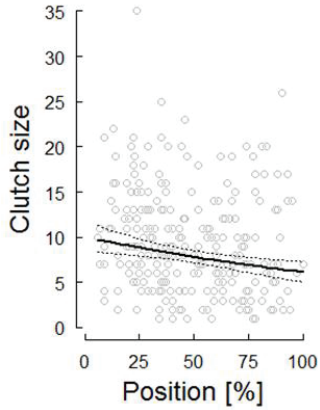


Figure S2: Oviposition occurred more frequently at lower positions on the shoot (0% = ground level) for all conspecific density treatments. Circles show the original data, and the lines indicate the model predictions with bootstrapped confidence limits.

Table S1: Oviposition depending on vertical position on the shoot and treatment.

Model Type	Random factor	Response Variable	Explanatory Variables	χ^2	Df	AIC	$R^2_{\text{GLMM(m)}}$	$R^2_{\text{GLMM(c)}}$	p-value
GLMM (Poisson)	Plant/Obs	Clutch size	intercept	456.86	1	1565.70	0.07	0.09	<0.001
			T	11.46	2	1565.70	0.07	0.09	<0.01
			P.rel	9.54	1	1565.70	0.07	0.09	<0.001
			$T \times P.rel$	2.30	2	1567.42	0.07	0.09	0.31

