THE DISTRIBUTION OF LEAF BEETLES ON MULTIPLE SPATIAL SCALES: CAUSES AND CONSEQUENCES

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‘Freude am Schauen und Begreifen
ist die schönste Gabe der Natur’

Albert Einstein

Table of Contents

Chapter 1

General Introduction

1.1 INTRODUCTION

Herbivorous insects, making up more than one quarter of all macroscopic organisms, are the major link between the primary producers – the green plants – and a multitude of animals at higher trophic levels (Bernays & Chapman, 1994). Although the distribution of herbivores within the 'green world' as well as their interactions with natural enemies have been the subject of a plethora of studies and textbooks (e.g. Lawton & McNeill, 1979; Price *et al.*, 1980; Crawley, 1983; Bernays & Chapman, 1994; Olff *et al.*, 1999; Dicke, 2000; Walker & Jones, 2001; Herrera & Pellmyr, 2002), we are still far from a thorough understanding of the causes and consequences of herbivore distribution patterns.

The unifying theory behind all patterns and processes is the concept of natural selection as the driving force of adaptive evolution (Darwin, 1859). Only in believing that all behavioral traits of animals underlie evolutionary processes and are shaped by different selection pressures, we can understand both *proximate* (i.e. ontogenetic and mechanistic) and *ultimate* (i.e. phylogenetic and adaptive) reasons for the realized distribution patterns on any spatial scale. In the light of evolution, it is thus more informative to analyze patterns of herbivore egg distribution instead of distribution of adults, as we may not always know why a herbivore dwells in a certain place at the time of our observation, however, we might infer that female oviposition site choice is subject to adaptive evolution.

Egg clutch distributions can be investigated on different spatial scales: We can ask where they are found in the landscape, where inside the habitat, on which plants within the habitat, and finally, where on the plant? Scaling down from fragmented landscapes to individual plants, this introduction will give an overview on the potential causes and consequences of herbivore egg distribution patterns.

Across spatial scales, different processes (fitness considerations, physiological and sensory abilities, population dynamics, dispersal behavior, the history of the landscape, etc.) as well as biotic and abiotic environmental factors are supposed to shape the distribution of herbivores (Crawley, 1983; Bernays & Chapman, 1994; Hanski & Gaggiotti, 2004; Krebs, 2001). Generally, the egg distribution of phytophagous insects will be influenced by the distribution of suitable host plants on any spatial scale (Bernays & Chapman, 1994). Still, egg clutches are neither deposited on every patch that contains suitable host plants nor on every host plant within a suitable patch.

On the landscape scale (subsection 1.1.1), the realized distribution pattern will be most likely determined by processes like dispersal, population and metapopulation dynamics, as well as by the history of the landscape (Hanski & Gaggiotti, 2004). On smaller spatial scales like the microhabitat (subsection 1.1.2), the individual host plant (subsection 1.1.3), and the microsite within a plant (subsection 1.1.4), the realized egg distribution pattern will depend both on proximate factors, i.e. on the insects' ability to find, to recognize, and to assess the quality of the oviposition site (Crawley, 1983; Bernays & Chapman, 1994; Bernays & Wcislo, 1994), and on ultimate factors, i.e. on the fitness consequences of oviposition site choice (Jaenike, 1978; Crawley, 1983; Thompson, 1988; Bernays & Chapman, 1994), which can also be altered by the impact of natural enemies (Jeffries & Lawton, 1984).

The importance of different proximate and ultimate factors may change with spatial scale (Heads & Lawton, 1983; Williams *et al.*, 2001; Tscharntke & Brandl, 2004) and there are various possibilities of interactions between the different spatial scales. For example, microhabitat and oviposition plant choice are supposed to be closely interconnected, as microhabitat features may influence herbivore fitness directly and indirectly (by influencing plant characteristics). But also population dynamics, dispersal between populations, and changes in the landscape, which are primarily supposed to affect the distribution of herbivores on the landscape scale, add further variability to the fitness consequences of distribution patterns within habitat patches. For example, changes in population density due to dispersal events or changes in habitat quality resulting from shifts in land use or from succession may modify the fitness consequences of oviposition strategies on a temporal scale.

An important question in this context is: how to quantify individual fitness? According to Krebs (2001), fitness is '*a measure of the contribution of an individual to future generations and can also be called adaptive value. Individuals have a higher fitness if they leave more descendants.*' He further states that '*individuals can be fitter for three reasons: They may reproduce at a high rate, they may survive longer, or both.*' Consequently, fitness should ideally be quantified across several generations and should include not only the information how many offspring an individual produces but how many of them survive, and even better, how many of them successfully reproduce themselves. However, such comprehensive fitness measures are difficult to obtain. As a compromise, the fitness consequences of potential oviposition strategies of phytophagous insects are often analyzed indirectly. Female choice is replaced by examining female oviposition patterns of whole populations. Offspring performance and survival is then experimentally quantified on different types of suitable host plants. Finally it is analyzed whether offspring performance and survival was optimal on those types of plants that were preferred by females for oviposition. This approach (which has been reviewed by Mayhew, 1997) will also be used in the manuscripts presented in this thesis.

1.1.1 Where are herbivores found in the landscape?

On a landscape scale, herbivorous insects are, first of all, affected by the distribution of their host plant(s). Herbivores can only survive and reproduce where they find their hosts in a sufficient quantity and quality. Due to the accelerating loss and fragmentation of natural habitats in the last decades (Moore, 1962; Burgess *et al.*, 1981; Saunders *et al.*, 1991; Morris, 1995; Malanson, 1999; Fahrig, 2001; Hunter, 2002), herbivores are confronted with a highly diverse mosaic of patches that may either represent potential habitats (i.e. fulfill requirements for successful breeding) or not. Since less specific habitat requirements increase the amount of suitable habitat for a species, generalist herbivores may be less affected by habitat fragmentation than specialists (Lawton, 1995; Steffan-Dewenter, 2003; Biedermann, 2004). However, also polyphagous herbivores are expected to be affected by habitat fragmentation, as habitat loss is often substantial and generalists also suffer from habitat isolation.

As a consequence of habitat fragmentation becoming a subject of nature conservation policy, a large body of theory and empirical evidence has been developed on explaining the distribution of species within fragmented landscapes (Hanski & Gaggiotti, 2004), which can be summarized as *metapopulation* concept (introduced by Levins, 1969, 1970).

The term metapopulation describes a *'population of populations, with colonization and extinction of local populations in a metapopulation likened to the births and deaths of individuals in a local population'* (Hanski & Gaggiotti, 2004), i.e. metapopulations are regionally connected populations of local populations inhabiting discrete patches that depend on the exchange of individuals between each other (Levins, 1970). Local populations have a substantial risk of extinction, but can also be (re-)colonized (Hanski & Gaggiotti, 2004). Therefore, long-term persistence of a metapopulation requires a balance across the landscape between local extinctions of individual populations and new colonizations of vacant habitat patches (Hanski, 1998; Moilanen & Hanski, 1998; Hanski & Gaggiotti, 2004).

In the first metapopulation concept (developed by Levins, 1969, 1970) all habitat patches were assumed to be of equal area and isolation, local populations had entirely independent dynamics, and dispersal rates were supposed to be so low that they did not influence local population dynamics. In contrast, the *incidence-function model* developed by Hanski (1994a,b) describes a spatially realistic modification of the Levins model. It allows that patches differ in area and have specific spatial locations. Moreover, it assumes a substantial rate of dispersal between patches. Based on these prerequisites, the occurrence probability (which results from the extinction and the colonization probability) of a species within a patch is supposed to be influenced by the size and carrying capacity of a patch, by its isolation, and by the occurrence and density of the species on neighboring patches.

Metapopulation theory predicts that the extinction probability of a species within a patch depends on patch size. Based on the idea that there is a *minimum viable population size*, i.e. a minimum number of individuals in a population is needed to maintain a substantial chance of surviving for a relatively long period of time (Soulé, 1980), large habitat fragments have a higher probability of keeping the population density above this threshold. On the one hand, large populations are less prone to extinction caused by demographic and/or environmental stochasticity (Goodman, 1987; Gabriel & Bürger, 1992). On the other hand, large populations have a more diverse gene pool which decreases the risk of extinction resulting from inbreeding, loss of genetic variability, and manifestations of negative mutations (Lande, 1994; Lynch *et al.*, 1995). Indeed, many field studies have shown that small populations in small habitat fragments have a high risk of extinction (e.g. Schoener & Spiller, 1987; Kindvall & Ahlén, 1992; Hanski, 1994a). Hanski & Gaggiotti (2004) assume that there exists a linear (Kindvall & Ahlén, 1992) or some other simple relationship (Hanski *et al.*, 1996) between patch size and local population size.

The quality of habitat fragments is not explicitly considered in classical metapopulation theory, but Hanski & Gaggiotti (2004) hypothesize that the lower extinction risk of populations in large habitat fragments may also be due to the greater heterogeneity of habitat quality in large patches compared to small patches. For example, habitat patches that are of a uniformly high quality under ‘typical’ weather conditions may greatly deteriorate in quality under ‘extreme’ conditions, while more heterogeneous patches may provide some high quality subareas at any time (Kindvall, 1996). More recently, Thomas *et al.* (2001) pointed out that also habitat quality itself (e.g. host plant density) can crucially alter the carrying capacity of a patch (Clarke *et al.*, 1997; Dennis & Eales, 1997; Thomas *et al.*, 1998; Wiegand *et al.*, 1999) and may even be more important for species persistence within a patch than the mere size of the habitat fragment. Accordingly, they suggest to integrate patch quality into metapopulation theory, as patches of equal size but of differing habitat quality can differ substantially in their carrying capacity.

The colonization probability of a patch depends on the size and isolation of the patch, on the occurrence and density of the species on neighboring patches, as well as on the probability that immigrants can establish a viable population in the new patch. Colonization probability increases with an increasing number of immigrants arriving at the patch per unit time (Hanski & Gaggiotti, 2004). The absolute number of emigrants leaving the surrounding patches will increase with increasing size and/or population density of these patches. Furthermore, the fraction of dispersers that will reach a certain patch increases with increasing size of this patch (as it will be more easily found by the dispersers) and with increasing number of and decreasing distance to surrounding occupied patches (as the probability of dying during dispersal through the unfavorable matrix is supposed to increase with increasing dispersal distance). Finally, the probability that immigrants can establish a viable population will depend on the quality of the respective habitat fragment.

Habitat fragmentation, however, may not only have negative effects on metapopulation survival, but the *environmental heterogeneity* that results from fragmentation can even be beneficial for the stability of interacting metapopulation systems (Krebs, 2001). These effects have often been neglected, as the majority of studies focused on one-species systems. Huffaker *et al.* (1963) showed in an elegant laboratory experiment, consisting of two mite species living in a 252-orange universe with a complex series of barriers between the orange patches, that the metapopulation of prey mites survived, as it was able to colonize new oranges faster than the predatory mite species. Thus, the prey mites could establish a population on a newly colonized orange and escape to the next before the predatory mite arrived and destroyed the population again.

Additionally, natural enemies of herbivores are also affected by habitat fragmentation, depending on their mobility and on their degree of specificity (Kruess & Tscharntke, 1994; Holt, 1996). Both theoretical models (Holt, 1996) and empirical studies (e.g. Lei & Hanski, 1997; Roland & Taylor, 1995; Zabel & Tscharntke, 1998) suggest that higher trophic levels are more negatively affected by fragmentation than their hosts, as they are more hampered in their dispersal by habitat isolation and are often concentrated in smaller populations that are more prone to extinction by stochastic processes (Kruess & Tscharntke, 1994). Thus, isolated habitat patches that can be reached by e.g. a herbivore but not by its parasitoid may be used as refuges and contribute to metapopulation survival.

Therefore, higher trophic levels, i.e. natural enemies, should be included in metapopulation studies, as they can influence both the dynamics of populations (i.e. the probability of extinction) within habitat patches (Vandermeer & Carvajal, 2001; van Nouhuys & Hanski, 2002) as well as the dispersal mortality between patches (i.e. the colonization probability). Still, empirical studies examining the influence of natural enemies on metapopulation dynamics are rare (Ohsaki & Sato, 1990; van der Meijden & van der Veen-van Wijk, 1997; Esch *et al.*, 2005).

Despite extensive theoretical literature on metapopulation dynamics (Hanski & Gaggiotti, 2004 and references therein; Hovestadt *et al.*, 2000, 2001; Poethke & Hovestadt, 2002; Poethke *et al.*, 2003) and numerous empirical studies (Hanski & Gaggiotti, 2004 and references therein; Kindvall, 1999; Ricketts, 2001; Hein, 2004), our understanding of metapopulation dynamics in real fragmented landscapes – especially for multitrophic systems (Hunter, 2002; van Nouhuys & Hanski, 2002) – is still restricted (Harrison & Taylor, 1997), largely due to practical problems of conducting sound empirical research at a sufficiently large spatial scale.

What further complicates empirical studies on metapopulations is our limited knowledge on the influence of the matrix in between potential habitat patches on dispersal rates (Wiens *et al.*, 1997; Pither & Taylor, 1998; Ricketts, 2001). Connectivity measures like the distance to the *nearest neighbor* or even the *connectivity* measure developed by Hanski (1999), do not account for the structure of the matrix, which can have a considerable influence of the *effective isolation* of a potential habitat patch (Gustafson & Gardner, 1996; Ricketts, 2001; Goodwin & Fahrig, 2002). Although several field studies have been conducted in this context (Jonsen *et al.*, 2001; Ricketts, 2001; Goodwin & Fahrig, 2002; Hein *et al.*, 2003; Cronin, 2003; Haynes & Cronin, 2003; Hein *et al.*, 2004, 2005), the response to different matrix structures is still unknown for the majority of species.

1.1.2 Which microhabitats do herbivores choose within a habitat?

Scaling down one step, we can ask what determines the distribution of egg clutches within habitat patches, i.e. which microhabitats are chosen by for oviposition by females. As already described above (section 1.1), both physiological abilities of females and fitness considerations are supposed to shape microhabitat choice. Females are expected to choose microhabitats for oviposition that enhance the performance and survival of their offspring according to the *preference-performance hypothesis* (Thompson, 1988; Thompson & Pellmyr, 1991). However, they would have to detect and to reach all available microhabitats within a habitat patch and to evaluate their quality in order to distribute their egg clutches optimally. Thus, the realized egg distribution will only be a rough approximation of the theoretical optimum and can only be optimal with regard to all microhabitats that were actually evaluated by the female.

As a first determinant of microhabitat choice, the distribution of host plants and/or structures onto which egg clutches are deposited within the habitat represents the basis for any possible egg distribution pattern (Bernays & Chapman, 1994), i.e. even the ‘best’ microhabitat will not be chosen, when it does not contain suitable plants or structures for oviposition. The *ideal free distribution* (Fretwell & Lucas, 1970; Milinski & Parker, 1991) predicts that herbivores distribute themselves (or their egg clutches) in order to utilize resources optimally, i.e. more herbivores/eggs aggregate in areas of high resource density than in areas of lower resource availability. However, empirical studies propose several reasons (host plant detection ability, influence of natural enemies, microclimate) why herbivores may deviate from the ideal free distribution pattern (e.g. Kennedy & Gray, 1993; Pulido & Díaz, 1997; Williams *et al.*, 2001).

The ability to detect host plants from a distance, which may be accomplished via visual and/or olfactory cues, plays a key role in microhabitat choice both in species that oviposit onto host plants or on non-hosts, as also non-host plants that are used for oviposition should be located next to a sufficiently large number of host plants. However, perceptual constraints may prevent herbivore females from detecting their host plant or from assessing host plant density. Although the utilization of vision in host plant location has been described for some herbivorous beetles (Tanton, 1977; Hausmann *et al.*, 2004), the importance of visual stimuli still needs to be analyzed in the majority of species. Potential optical plant features which could be used for host location are color, size, and shape. Recent studies show that certain herbivores can be very sensitive to plant color, i.e. especially to the spectral composition of leaves (Bullas-Appleton *et al.*, 2004; Fischer *et al.*, 2004). Plant size and shape, by contrast, might be less reliable cues for host plant identification, as individuals of a plant species often vary in size and shape already within a site, even more from site to site, and from season to season (Prokopy & Owens, 1983). Moreover, dense vegetation can hamper visual host plant detection from a distance (Rausher, 1981), especially in beetles that rather walk than fly.

Presumably, olfactory cues are more reliable signals for host plant recognition and may also be used to assess host plant density. Olfactory cues may facilitate host plant location even in a complex environment, if the insect central nervous system receives the volatile information at a fine-scale spatio-temporal resolution (Held *et al.*, 2003; Bruce *et al.*, 2005). In laboratory experiments, many beetle species are able to identify their host plant based on olfactory cues (e.g. Bartlet *et al.*, 1997; Müller & Hilker, 2000; Kalberer *et al.*, 2001; Zhang & Schlyter, 2004; Kalberer *et al.*, 2005) either *via* species-specific compounds (e.g. Feeny, 1970; Blight *et al.*, 1995; Bartlet *et al.*, 1997) or specific ratios of ubiquitous compounds (e.g. Visser & Avé, 1978; Barata *et al.*, 2000; van Tol & Visser, 2002), the latter scenario being in general more common (Bruce *et al.*, 2005). However, due to air turbulences, relevant concentration gradients of plant odors are unlikely to exist more than a few centimeters away from a plant, i.e. at distances beyond, the insect cannot follow an odor gradient to its source because there is no gradient to follow (Bernays & Chapman, 1994). This emphasizes the importance of studies under more natural conditions that verify the relevance of results obtained in the laboratory under field conditions. Such studies are, however, rare (Guerin *et al.*, 1983; Morrow *et al.*, 1989; Freund & Olmstead, 2000; Finlay-Doney & Walter, 2005; Bengtsson *et al.*, 2006).

If we assume the extreme case that the female can recognize her host plant only after contact, she has to scan her habitat randomly for host plants. Supposing that she will oviposit on any suitable plant that she meets, the resulting distribution pattern will deviate from the ideal free distribution: Plants growing singly or in areas of low host plant density will receive disproportionately more eggs than plants in areas of high host plant density, as these plants have a higher probability of being found by the female (Cain, 1985). Also egg- and time-limitations may influence egg distribution patterns within the habitat (West & Cunningham, 2002). If egg load is high, if females are time-limited, and if traveling from plant to plant is costly, they are supposed to oviposit more eggs on isolated host plants than on aggregated ones. This results from the fact, that plants in areas of low density should be more readily accepted for oviposition as females have a reduced probability of finding other host plants nearby in the available time.

Regarding fitness considerations, several microhabitat properties may influence offspring development and survival. From a bottom-up perspective, areas of high host plant density are supposed to enhance the survival chances of offspring, as larvae may more easily change from one host plant to another when food quality declines. In polyphagous species, also the plant composition of the microhabitat may be important, as mixed diets may enhance larval development more than e.g. a high availability of the primary host plant (Bernays *et al.*, 1994; Pfisterer *et al.*, 2003).

Top-down influences may change egg distribution patterns both directly and indirectly. Directly, they may have an effect, for example, if natural enemies preferentially attack patches of high or low herbivore density (Hassell, 1980; Crawley, 1983; Jones & Hassell, 1988). Indirectly, they may induce herbivores to alter their distribution pattern in order to avoid enemy attack, i.e. to obtain *enemy-free space* (Jeffries & Lawton, 1984; Hawkins *et al.*, 1993; Hopkins & Dixon, 1997). The resulting pattern will most likely deviate from the ideal free distribution as refuges rarely coincide with places of highest host plant density or best quality resources (Williams *et al.*, 2001).

Enemy-free space can not only be gained by ovipositing in areas of low egg density, but also the complexity of the microhabitat, both structurally and chemically, has only recently been recognized as possible source of enemy-free space for herbivores. Predators and parasitoids (primarily those that search their host by walking and recognize it only after contact) may be impeded from finding the herbivore by a dense and structurally complex non-host vegetation, simply due to the increased search effort (Sheenan, 1986). The searching efficiency of both walking and flying enemies that recognize their host from a distance using olfactory cues may be reduced in chemically complex environments, i.e. in microhabitats with a diverse mixture of non-host plants (Tahvanainen & Root, 1972; Uvah & Coaker, 1984; Vandermeer, 1989).

Another factor that is supposed to critically influence microhabitat choice within habitat patches is microclimate (Bach, 1984; Sipura & Tahvanainen, 2000; Sipura *et al.*, 2002). Depending on the species under study, sunny (Sipura & Tahvanainen, 2000), shady (Rausher, 1979), moist (Sipura *et al.*, 2002), or dry microhabitats may be chosen for oviposition. While moist and shaded areas may be preferred to avoid desiccation, sunny microhabitats are supposed to enhance all developmental processes in growing insects (Grossmueller & Lederhouse, 1985; King *et al.*, 1985; Jackson & Elliott, 1988; Stiefel *et al.*, 1997)

1.1.3 On which plants do herbivores oviposit?

Once the female has chosen a certain microhabitat, she may further decide, which individual plant within this microhabitat she will use for oviposition. In species that oviposit directly on a host plant, this poses two problems for the female: First, she has to confirm whether the plant indeed belongs to the right species and, second, she should assess its quality (Bernays & Chapman, 1994). In species that do not oviposit on host plants, the identity and quality of the oviposition plant itself may not be important, but the oviposition decision may be shaped by other plant properties (e.g. plant structure). In any case, oviposition plant choice will be influenced both by the physiological and sensory capabilities of the female and by fitness considerations regarding offspring performance.

In species that oviposit on host plants, the female may use visual, olfactory, and contact cues or different combinations of all three for host plant identification, similar to host plant detection from a distance (cf. subsection 1.1.2). From these three types of cues, contact cues are supposed to be most reliable. Already plant cuticular waxes can be used to decide on host plant acceptance (Eigenbrode & Espelie, 1995; Müller & Riederer, 2005). Furthermore, after test-biting individual host-specific compounds stimulate feeding in several specialist herbivores (Metcalf *et al.*, 1980; Tallamy & Krischik, 1989; Larsen *et al.*, 1992). In other species, a mixture of several compounds is required (Müller & Renwick, 2001; van Loon *et al.*, 2002), whose components may be inactive when offered individually, but show synergistic effects when offered together (Endo *et al.*, 2004; Tamura *et al.*, 2004).

After having identified a suitable host, the female has to decide whether to oviposit on that plant or not. As already mentioned above, the preference-performance hypothesis predicts that female oviposition preference should be positively correlated with offspring performance (Thompson, 1988; Thompson & Pellmyr, 1991). Regarding the question, which host plant features make a plant optimal for larval performance, the *plant stress hypothesis* (White, 1984; Mattson & Haak, 1987) was developed as a first, more specific hypothesis on oviposition strategies. It suggests that females should oviposit on stressed plants, as stress causes an increase in the amount of nitrogen available in the plant tissues and a decrease in the synthesis of defensive chemicals (White, 1984). Both makes those plants a better source of food for young herbivores. By contrast, the *plant vigor hypothesis* (Price *et al.*, 1987; Price, 1991) predicts that females should choose large, vigorously growing host plants for oviposition, using the same arguments for vigorous plants as used by White (1984) for stressed plants.

Studies examining these two hypotheses (plant stress and plant vigor), however, have yielded mixed results (e.g. Larsson, 1989; Waring & Cobb, 1992; De Bruyn, 1995; Preszler & Price, 1995; Meyer & Root, 1996; Inbar *et al.*, 2001). While there are many species in which females seemed to choose host plants for oviposition that were optimal for offspring development, there are quite a lot species where female preference did not coincide with optimal offspring performance.

There are several possible explanations for a lack of positive correlation between female preference and offspring performance. On the one hand, the female may not be able to assess the quality of the host plant properly. For example, the *neural limitation hypothesis* (Levins & MacArthur, 1969) predicts that neural constraints result in a trade-off between diet breadth and the ability to discriminate among hosts of different quality, i.e. polyphagous herbivores may not be very well able to assess the quality of all their host plant species. On the other hand, a female can also enhance her fitness by feeding on high quality hosts herself, which may increase the number of eggs she can produce (cf. the definition of fitness in section 1.1). As optimal adult and optimal offspring resources can differ and be separated in space, search-time constraints may prevent the optimization of both strategies (Nylin & Janz, 1996; Krebs & Davies, 1997). Thus, Scheirs & De Bruyn (2002) suggested to evaluate the oviposition strategy of a female not only in terms of offspring performance but also by analyzing where a female feeds, whether she preferentially oviposits on her food plants, and finally, whether her oviposition strategy increases her overall fitness.

Host plant choice that seems to be suboptimal in terms of quality may also be influenced by higher trophic levels, as nutritionally inferior host plants may be chosen when they provide enemy-free space for the offspring (Hawkins *et al.*, 1993; Björkman *et al.*, 1997; Ballabeni *et al.*, 2001; Obermaier *et al.*, 2001; Singer & Stireman, 2003). Enemy-free space may even be more important for species that do not oviposit on host plants. For example, non-hosts may be chosen for oviposition as the occurrence of egg clutches may be less predictable on non-host plants, i.e. parasitoids and predators may not as easily associate plant cues with the possibility of egg occurrence. These plants may then be chosen based on criteria like stability or structural complexity, as complex plant structures may hamper host finding e.g. by parasitoids (Andow, 1990; Lukianchuk & Smith, 1997; Gingras *et al.*, 2002; Andow & Olson, 2003; Gingras *et al.*, 2003). In species that hibernate in the egg stage, the stability of the whole oviposition plant may be crucial for the survival probability of the egg clutch.

1.1.4 *Where on the plant does oviposition occur?*

Even when a female has decided to oviposit on a certain plant individual, she may further select where exactly on that plant she will place the egg clutch. Also on this spatial scale, the decision of the female will be shaped by the questions which oviposition site will optimize her fitness and whether she is able to comprehensively evaluate all possible oviposition sites with regard to the resulting fitness consequences.

In case the oviposition plant is a host plant, the female may place her eggs on the most nutritious plant parts (e.g. on the youngest leaves), or even better on those plant parts that will be most nutritious when the larvae hatch (Mattson, 1980; Awmack & Leather, 2002). Another important feature of an optimal oviposition site within a host plant may be the stability of the substrate on which the egg clutch is placed, e.g. egg clutches that are deposited onto plant leaves may be placed next to the mid-rib. Furthermore, in addition to choosing a host plant growing in a suitable microclimate (cf. subsection 1.1.2), also different parts of the oviposition plant may provide diverse microhabitats for optimal egg development. Depending on the herbivore species and on the time of year when oviposition occurs either sunny, shady, moist, or dry microclimates may be chosen within the plant.

Also within the plant, top-down influences may shape the decision where to oviposit. Both theoretical and empirical studies show that host finding by parasitoids can be impaired on complex structured plants (either host or non-host plants) (Andow, 1990; Lukianchuk & Smith, 1997; Gingras *et al.*, 2002; Andow & Olson, 2003; Gingras *et al.*, 2003). In addition to choosing complex plant individuals (cf. subsection 1.1.3) females may further select the most complex sub-part of the plant. This seems to be most important in species whose natural enemies search for their hosts mainly by walking.

1.2 SCOPE AND OUTLINE OF THE THESIS

Within the theoretical framework presented above, this thesis investigates the distribution of egg clutches of the specialized tortoise beetle *Cassida canaliculata* Laich. (Coleoptera: Chrysomelidae) on multiple spatial scales and compares it to that of the polyphagous tansy leaf beetle *Galeruca tanaceti* L. (Coleoptera: Chrysomelidae). *C. canaliculata* was chosen, as it is strictly monophagous on meadow sage, *Salvia pratensis* L. (Lamiales: Lamiaceae) (Wencker & Silbermann, 1866; Bourgeois & Scherdlin, 1899; Reitter, 1912; Graser, 1984; Trautner *et al.*, 1989; A. Heisswolf and D. Gabler, unpublished data), i.e. it does not even feed on the closely related *S. officinalis* (A. Heisswolf and D. Gabler, personal observations). Moreover, it also oviposits exclusively on its single host plant species. *G. tanaceti*, in contrast, feeds on species of many plant families and does only rarely oviposit on host plants, but most often on dry grass stalks.

Based on the results of an earlier study (Heisswolf *et al.*, in press), the present thesis was designed to examine the distribution pattern of *C. canaliculata* scaling down from fragmented landscapes to individual host plants. It analyzes (a) which habitats are optimal for oviposition by *C. canaliculata* within the fragmented landscape of the nature reserve 'Hohe Wann', (b) by which means *C. canaliculata* is able to find its host plant *S. pratensis* within the habitat, (c) the consequences of the egg distribution pattern on the host plant scale on larval development and survival, and (d) whether the reproductive strategies of the monophagous *C. canaliculata* differ from that of the polyphagous leaf beetle *Galeruca tanaceti* L. (Coleoptera: Chrysomelidae).

Prior to the presentation of the different scientific manuscripts, ***Chapter 2*** gives a more detailed description of the main study system around the specialist *C. canaliculata*, the comparative system about the generalist *G. tanaceti*, and the study area, the nature reserve 'Hohe Wann', in which both tritrophic systems occur.

Chapter 3 analyzes the important features of optimal habitat patches for oviposition by *C. canaliculata* and its egg parasitoid *Foersterella reptans* Nees (Hymenoptera: Tetracampidae) within the nature reserve 'Hohe Wann' by means of quantitative habitat models. ***Chapter 4*** presents the results of a detailed laboratory study on the host plant finding process of *C. canaliculata*, in which the importance of olfactory and contact cues was analyzed with various methods. As the olfactory bioassays yielded equivocal results, a semi-natural arena was designed in which the movement pattern of *C. canaliculata* was analyzed depending on whether a host plant, a non-host plant, or no plant at all were presented in the center of the arena *(**Chapter 5**)*. In ***Chapter 6***, the development and survival of *C. canaliculata* larvae depending on host plant size, i.e. the fitness consequences of a female oviposition preference for large host plants, were studied both in the laboratory and in a predator-exclusion experiment in the field. Finally, ***Chapter 7*** & ***Chapter 8*** present studies on the egg distribution patterns of the polyphagous tansy leaf beetle *Galeruca tanaceti* L. (Coleoptera: Chrysomelidae).

Chapter 2

Study systems and study area

2.1 THE SPECIALIST SYSTEM

The monophagous tortoise beetle *Cassida canaliculata* Laich. (Figure 2.1a) belongs to the family of leaf beetles (Coleoptera: Chrysomelidae, subfamily Cassidinae). In central Europe approximately 25 species of the genus *Cassida* are known. With a body size of 9–11 mm *C. canaliculata* is the largest German tortoise beetle (Trautner *et al.*, 1989). Within Germany, it is endangered, in Bavaria it is even threatened by extinction (Kippenberg, 2003). However, on warm slopes within the study area, the nature reserve 'Hohe Wann' (cf. section 2.3), it is locally abundant (A. Heisswolf, E. Obermaier, and S. Reichmann, personal observations).

As can already be guessed from the name 'tortoise' beetle, pronotum and elytrae cover the whole body in these species, i.e. when the beetle is not moving neither antennae nor legs are visible. Both pronotum and elytrae are green to auburn colored, erratically covered with brown spots, and shimmering golden. Moreover, the edges of pronotum and elytrae are curved and brown, which – altogether – makes *C. canaliculata* easy to identify (Figure 2.1a). Until now, little has been published on the ecology of *C. canaliculata* (Steinhausen, 1949; Trautner *et al.*, 1989; Heisswolf *et al.*, 2005, in press), i.e. the majority of information on oviposition and larval development that is described below results from personal observations.

Oviposition occurs in small clutches of 4-16 eggs exclusively on the host plant species *Salvia pratensis* L. (Lamiales: Lamiaceae, Figure 2.2a). Egg clutches (Figure 2.1b) are attached to the abaxial side of the leaf starting with a secretion layer onto which the eggs are deposited in several strata which are also separated by secretion layers. A final layer, which hardens and darkens within some hours after oviposition, covers the whole egg clutch and makes it physically less accessible to predators (cf. Damman & Cappucino, 1991). Although the chemical composition of this secretion has not yet been analyzed in *Cassida canaliculata*, chemical protection of egg clutches is widespread in leaf beetles (Hilker & Meiners, 2002a).

Oviposition takes place from April to early June and each female produces 20–60 egg clutches. Larvae (Figure 2.1c) hatch after approximately 10 days and pupate 25–50 days later (Figure 2.1d), depending on temperature. All larval stages feed on the abaxial side of the host plant leaves. Like in other *Cassida* species, the larvae carry a so-called 'fecal shield' which consists of old exuviae and feces (Figure 2.1c). The function of this shield has been studied in several species of the Cassidinae subfamily (Olmstead, 1996; Müller & Hilker, 1999). Generally, it is assumed, that it acts as a repellent to predators and parasitoids (Olmstead, 1996). However, whether this is also true for *C. canaliculata* remains to be analyzed as e.g. Müller & Hilker (1999) and Schaffner & Müller (2001) found an attractive effect of the fecal shield on both predators and

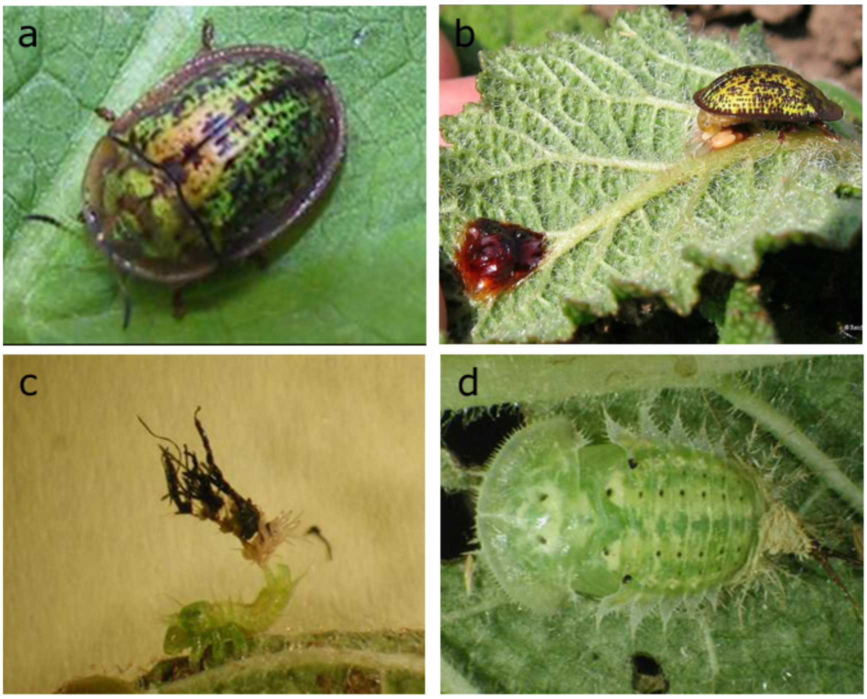

Figure 2.1: *Cassida canaliculata* Laich. (Coleoptera: Chrysomelidae): a) adult beetle, b) egg clutch and egg laying female, c) larva with fecal shield, d) pupa. Photographs by Dirk Gabler (a), Stefanie Reichmann (b), and Erhard Strohm (c + d).

parasitoids in several *Cassida* species.

Pupal development takes another 10-11 days and pupae (Figure 2.1d) sometimes can also be found attached to other plants than *S. pratensis* in the field. After hatching the young beetles stay for some weeks in their natural habitat prior to hibernation. Kosior & Klein (1970) describe for four other *Cassida* species a migration to nearby forests for hibernation, however, whether this is also the case in *C. canaliculata* is unclear. At least, the majority of patches with *C. canaliculata* occurrence in the nature reserve 'Hohe Wann' are adjacent to hedges and forests, which could be possible hibernation sites of *C. canaliculata*.

The only host plant of *C. canaliculata*, the meadow sage, *Salvia pratensis* L. (Lamiales: Lamiaceae, Figure 2.2) is a thermophilic species that usually grows on dry meadows and field edges, preferably on calcareous soils (Schmeil & Fitschen, 1996). The meadow sage is a perennial herb with a ground rosette (which can consist of several 'daughter rosettes') and one to several flower stalks that can reach a total height of up to 60 cm (Schmeil & Fitschen, 1996). The rosettes start to grow in late March, flowering begins in May and can last until August (Schmeil & Fitschen, 1996). The inflorescences of *S. pratensis* are arranged in whorls (Figure 2.2b). Usually, the species has six flowers per whorl and each flower contains four ovules. The seeds, which are

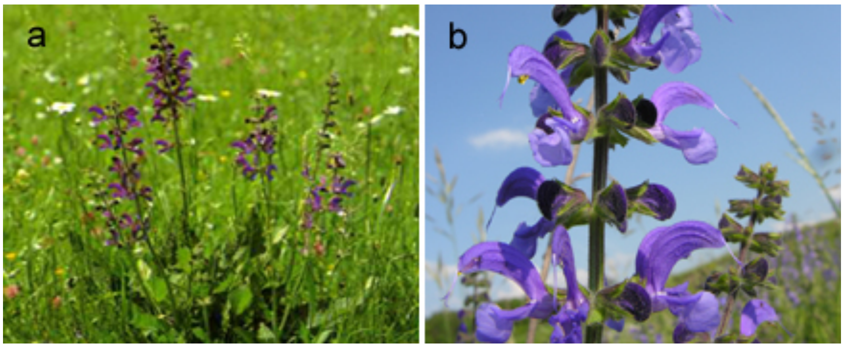

Figure 2.2: *Salvia pratensis* L. (Lamiales: Lamiaceae). (a) Flowering plant with several 'daughter rosettes' and (b) generative stalk with several flower whorls. Photographs by A. Baccarini (a) and Adelheid Burghardt (b).

released from mid-July onwards, germinate soon after being released if moisture is sufficient (Ouborg & van Treuren, 1995). They are assumed to disperse only over short distances (Ouborg & van Treuren, 1995). Seedlings need 4-5 years to reach flowering size (Hegland *et al.*, 2001). There are no reliable records of the life span of *S. pratensis*, but demographic data suggest that the average lifetime of an adult plant may range to a couple of decades (Ouborg & van Treuren, 1995).

The hymenopteran wasp *Foersterella reptans* Nees (Hymenoptera: Tetracampidae, Figure 2.3) is the only known egg parasitoid of *C. canaliculata* (S. Vidal, personal communication). It belongs to the Tetracampidae, which are a worldwide distributed family of the chalcid wasps (Chalcidoidea) and consist of approximately 50 species in 15 genera. Most chalcid wasps are parasitoids of other insects, attacking the egg or larval stage of their host, but there are also some phytophagous species.

F. reptans (formerly also known as *F. flavipes* Förster) parasitizes the eggs of several *Cassida* species (Labeyrie, 1962; Herting, 1973; Heisswolf *et al.*, in press), but

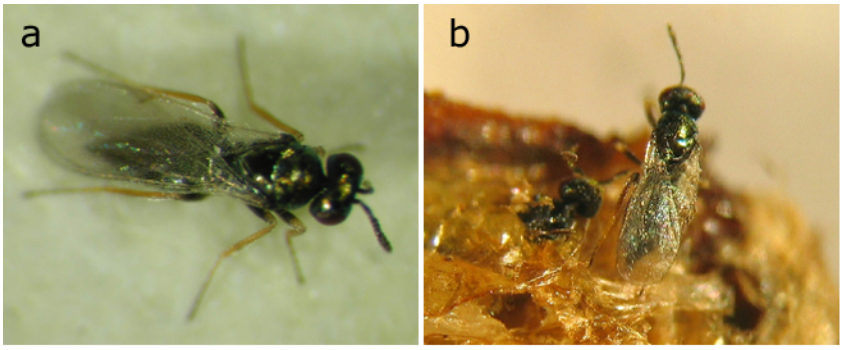

Figure 2.3: *Foersterella reptans* Nees (Hymenoptera: Tetracampidae). (a) Adult and (b) newly emerging adult. Photographs by Erhard Strohm (a) and Stefanie Reichmann (b).

also the larvae of *C. rubiginosa* (Bacher & Luder, 2005). It is a tiny (body length: 1-3 mm), dark-coloured wasp with a metallic blue-green shimmer. It can be recognized as chalcid wasp by the characteristically reduced wing venation (Bouček, 1958) and as *F. reptans* by its yellow coloured legs (which is also reflected in the former Latin species name '*flavipes*').

Although the oviposition behavior of *F. reptans* is not yet described in the literature, it can be deduced from other parasitoid species that egg clutches, which are covered by a secretion layer (like in *C. canaliculata*), are vulnerable to parasitism only for a relatively short time (several days), as the egg cover hardens very soon (reviewed by Gross, 1993; Hilker & Meiners, 2002b). Female parasitoids lay one egg per host egg and up to all eggs per clutch can be parasitized (A. Heisswolf, personal observations). From other Tetracampidae species, egg-adult development times of 10-30 days are reported (Murphy & LaSalle, 1999). After this time, the parasitoids emerge as adults from the host's egg clutch (Figure 2.3b). How and where the parasitoids hibernate is unexplored as well.

2.2 THE GENERALIST SYSTEM

In contrast to *C. canaliculata*, the polyphagous tansy leaf beetle *Galeruca tanaceti* L. (Coleoptera: Chrysomelidae, Figure 2.4a) feeds on species of the families Asteraceae, Brassicaceae, Caryophyllaceae, Dipsacaceae, Liliaceae, Lamiaceae, Polygonaceae, and Solanaceae (Lühmann, 1939; Prevett, 1953; Obermaier & Zwölfer, 1999). In the study area, the main host of *G. tanaceti* is *A. millefolium* L. (Asterales: Asteraceae, Figure 2.5b) (Meiners & Obermaier, 2004), but larvae can also be found feeding on *Tanacetum vulgare* L. (Asterales: Asteraceae, Figure 2.5a) (Meiners *et al.*, 1997) and quite frequently on *S. pratensis* (A. Heisswolf, B. Randlkofer, and E. Obermaier, personal observations).

Oviposition occurs in autum (September-December) on dry stalks of grasses and herbs (mostly non-hosts) (Prevett, 1953; Siew, 1966; Meiners & Obermaier, 2004; Obermaier *et al.*, in press). An egg clutch contains on average 60 eggs (Meiners *et al.*, 1997; Obermaier *et al.*, in press). Like in *C. canaliculata* the eggs are covered by a secretion layer which hardens and darkens within hours after oviposition. In *G. tanaceti*, both eggs and larvae contain anthraquinones (Hilker & Köpf, 1994; Meiners *et al.*, 1997), which may act as a defensive agent against natural enemies.

The egg clutches (Figure 2.4b) hibernate on the dry plant structures and can stay there for up to 7 months until the larvae (Figure 2.4c) hatch from March to May (Meiners *et al.*, 1997; Obermaier & Zwölfer, 1999). After pupation, the adults can be found from early June onwards before they enter a reproductive diapause in mid-summer.

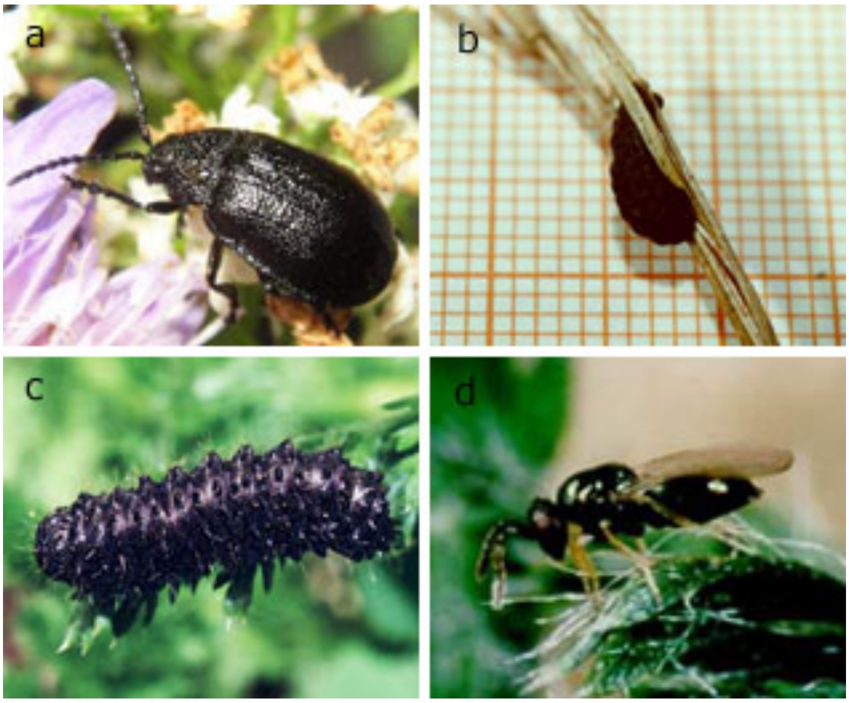

Figure 2.4: *Galeruca tanaceti* L. (Coleoptera: Chrysomelidae). (a) Adult, (b) egg clutch, (c) larva, and (d) egg parasitoid *Oomyzus galerucivorus* Hedqvist (Hymenoptera: Eulophidae). Photographs by F. Köhler (a), Barbara Randlkofer (b), zooex.baikal.ru (c), and Torsten Meiners (d).

The egg clutches of *G. tanaceti* are heavily parasitized by the eulophid wasp *Oomyzus galerucivorus* Hedqvist (Hymenoptera: Eulophidae, Figure 2.4d) (Meiners *et al.*, 1997; Meiners & Obermaier, 2004; Obermaier *et al.*, in press). Like *F. reptans*, *O. galerucivorus* belongs to the superfamily of chalcid wasps (Chalcidoidea). The Eulophidae are the largest and best studied family of the chalcid wasps, which consists of more than 3000 known species worldwide. With a body length of 1.5 mm *O. galeru-*

Figure 2.5: Host plants of *Galeruca tanaceti*. (a) Tansy, *Tanacetum vulgare* L. (Asterales: Asteraceae), and (b) yarrow, *Achillea millefolium* L. (Asterales: Asteraceae). Photographs by Adelheid Burghardt (a) and Erika Gussmann (b).

civorus is as tiny as *F. reptans* and is also of similar body shape and color. *O. galerucivorus* parasitizes different *Galeruca* species (Sinacori & Mineo, 1993), however, its main host in Germany is the tansy leaf beetle (T. Meiners, personal communication). No other parasitoids could be found parasitizing eggs of *G. tanaceti* (T. Meiners, personal communication).

Like *F. reptans*, *O. galerucivorus* attacks the eggs of *G. tanaceti* a few days after they have been laid. The larvae hibernate inside the egg clutches and emerge as adults shortly before the beetle larvae in spring (Meiners *et al.*, 1997; Meiners & Obermaier, 2004). Since no alternative hosts of *O. galerucivorus* are known within the study area, the adult parasitoids have to endure and probably enter a diapause during summer until oviposition of their host starts in fall (Meiners & Obermaier, 2004).

2.3 STUDY AREA – THE NATURE RESERVE 'HOHE WANN'

All field studies were conducted in the nature reserve 'Hohe Wann' in Northern Bavaria, Germany (50°0´N, 10°35´E). The nature reserve is part of the southwestern 'Hassbergetrauf'. It extends from Zeil a. M. in the south to Königsberg i. By. in the north (largest NS-extension: ca. 10 km) and from the 'Kapellenberg' in the east to Prappach in the west (largest EW-extension: ca. 4 km). The size of the nature reserve is approximately 1000 ha. Altitudes of the study area range from 238 to 388 m above sea level.

The 'Hassberge' represent the northern part of the Franconian 'Schichtstufenland'. This formation was deposited during the Triassic and the Jurassic. The most common types of rock are clay rock, clay, clay marl, clay slates, and marl slates that are interspersed with sands or carbonates. Also common are differently bound sandstones (Emmert, 1964; in Elsner, 1994).

The nature reserve 'Hohe Wann' is located in the climatic region 'Obermain' at

Figure 2.6: The nature reserve 'Hohe Wann'. Photographs by Annette Heisswolf.

the edge of the river Main valley, which is a transition zone between oceanic and continental climate. It is a typical area of summer rain as can be found also in other river valleys. Mean annual precipitation is 650-700 mm and mean annual temperature is 7.5-8.5 °C. The vegetation period (i.e. the period of a mean daily air temperature above 10 °C) lasts 150-160 days (Elsner, 1994).

Resulting from its complex geology and geomorphology as well as from a variety of management types, the nature reserve 'Hohe Wann' consists of a complex mosaic of different vegetation types (Figure 2.6). This vegetational complexity is still increased by succession processes that proceed as parts of the area are no longer managed (Elsner, 1994).

The most characteristic features of the nature reserve are small, mesoxerophytic grassland sites, which were formerly used as vineyards or pastures (Elsner, 1994; Figure 2.6). These patches are scattered between areas of different agricultural use. On flat areas mainly crop fields can be found, while the slopes are used as grassland or lay fallow and thus overgrow by shrubs. Semi-arid meadows like mesoxerophytic grasslands are most often found on south- to south-west faced slopes, while north exposed hillsides are often wooded (Hein, 2004).

During the MOSAIK-project (a collaboration between several German Universities, funded by the German Federal Ministry of Education and Research), which studied the influence of different management regimes on the survival of plant and animal species within the fragmented landscape of the nature reserve 'Hohe Wann', both a biotope type map (J. Eibich, unpublished data) and a digital landscape model (Schröder & Reineking, 2004; Rudner *et al.*, in press; Schröder *et al.*, submitted) were established for the whole nature reserve. As the Field Station Fabrikschleichach of the University of Würzburg participated in the MOSAIK-project, this information could also be used for the analysis of the distribution and survival of *C. canaliculata* within this thesis.

Chapter 3

Habitat size, isolation, and quality determine the distribution of a monophagous leaf beetle and its egg parasitoid in a fragmented landscape

with Stefanie Reichmann, Hans Joachim Poethke, Boris Schröder and Elisabeth Obermaier

SUBMITTED TO LANDSCAPE ECOLOGY

Abstract. Habitat fragmentation threatens the survival of many insect species. While ‘classical’ metapopulation theory stresses the importance of habitat size and isolation for species occurrence on a potential habitat patch, recent studies suggest that habitat quality is the missing third parameter in metapopulation dynamics, which may contribute more to species persistence than habitat size or isolation.

The present study analyzed the effects of habitat size, isolation, and quality for the occurrence and population density of the endangered specialized leaf beetle *Cassida canaliculata* Laich. (Coleoptera: Chrysomelidae), which is monophagous on meadow sage (*Salvia pratensis* L., Lamiales: Lamiaceae). Additionally, we analyzed whether any of these parameters also influenced the occurrence and density of the hymenopteran wasp *Foersterella reptans* Nees (Hymenoptera: Tetracampidae), which parasitizes the egg clutches of *C. canaliculata*.

Both size and isolation of habitat patches strongly determined the occurrence of *C. canaliculata*, while the population density increased with both habitat size and host plant density. The egg parasitoid *F. reptans* was positively density dependent, i.e. the occurrence probability increased with increasing population density of *C. canaliculata*. However, none of the other studied parameters influenced parasitoid occurrence or density.

In summary, the specialized herbivore *C. canaliculata* depended on large, un-isolated patches with high host plant density, which stresses the importance of habitat quality as the missing third parameter in metapopulation dynamics. The occurrence of the parasitoid *F. reptans* seemed to depend only on the population density of the beetle.

3.1 INTRODUCTION

The persistence of potential habitats and as a consequence thereof the persistence of many animal and plant species is endangered by massive human interference in the environment (Saunders *et al.*, 1991; Kruess & Tscharntke, 1994; Malanson, 1999; Fahrig, 2001; Hunter, 2002). Species are often confronted with a fragmented landscape in which they can only survive on patches that meet their respective needs. In order to enhance the survival chances of endangered species, we need to analyze which patch features are most important for the respective species.

Within a landscape, potential habitat patches can differ in quality, size, and isolation. In the literature, discussion has been quite controversial, which of these factors might be the most important for the occurrence of a species within a potential habitat patch (Thomas, 1984; Dennis & Eales, 1997; Hanski & Singer, 2001; Tscharntke *et al.*, 2002; Hanski & Gaggiotti, 2004).

Metapopulation theory states that the populations of a species are interdependent within a landscape and that long-term persistence depends on a balance across the landscape between local extinctions of individual populations and new colonizations of vacant habitat patches (Hanski, 1998; Moilanen & Hanski, 1998; Hanski & Gaggiotti, 2004). Based on this theory, the occurrence probability of a species within a patch is supposed to increase with increasing patch size and decreasing isolation. While both patch size and isolation raise the probability that dispersing animals will find a patch, patch size has also a positive effect on the survival chance of a population within a patch, as larger patches may support larger populations.

However, several recent studies emphasized that in addition to the size also the quality of a habitat patch determines the carrying capacity of this patch and may thus increase the survival chance of a population (Clarke *et al.*, 1997; Dennis & Eales, 1997; Thomas *et al.*, 1998; Wiegand *et al.*, 1999). Consequently, Thomas *et al.* (2001) suggested that habitat quality is the missing third parameter in metapopulation dynamics. They hypothesized that habitat quality and spatial effects operate at different hierarchical levels within the same process: while patch size and isolation can be important for the (re-)colonization probability of a patch, habitat quality is supposed to contribute to species persistence within a patch.

In the present study we analyzed the importance of patch size, isolation, and habitat quality for the occurrence and population density of the specialized leaf beetle *Cassida canaliculata* Laich. (Coleoptera: Chrysomelidae) within the Hohe Wann nature reserve (Northern Bavaria, Germany; 50°03´N, 10°35´E). *C. canaliculata* is strictly monophagous on meadow sage (*Salvia pratensis* L., Lamiales: Lamiaceae) (Wencker & Silbermann, 1866; Bourgeois & Scherdlin, 1899; Reitter, 1912; Graser, 1984; Trautner *et al.*, 1989; A. Heisswolf and D. Gabler, unpublished data) and can thus only survive on patches where *S. pratensis* occurs. Within Germany, *C. canaliculata* is endangered, in Bavaria it is even threatened by extinction (Kippenberg, 2003); however, on warm slopes in the study area it is locally abundant (A. Heisswolf and E. Obermaier, personal observations). The Hohe Wann nature reserve is characterized by a patchwork of different habitat types due to the geological and geomorphological heterogeneity of the area, agricultural land use, and small-scale microclimatic differences resulting from different exposition, inclination, and land use (Elsner, 1994).

We tested the hypotheses that occurrence probability and population density of *C. canaliculata* will increase with increasing patch size, with decreasing patch isolation, and with increasing habitat quality. Moreover, as the egg clutches of *C. canaliculata* are parasitized by the hymenopteran wasp *Foersterella reptans* Nees (Hymenoptera: Tetracampidae), we further analyzed whether the occurrence and rate of parasitism are correlated with any of these parameters or with the egg clutch density of *C. canaliculata*.

3.2 MATERIAL AND METHODS

3.2.1 Study sites

Prior to the present study, the occurrence of *S. pratensis*, the only host plant of *C. canaliculata*, was recorded within the whole nature reserve and a habitat map for *S. pratensis* was developed (S. Reichmann, unpublished data), which consisted of 161 different potential habitat patches for *C. canaliculata*. Within these patches, 77 random points were distributed (max. one per patch) using the Geographical Information Software Arc View GIS 3.2 (ESRI, Redlands, California). In the field, we located the points using a portable GPS. At 60 of these points, we carried out a complete data collection (as described below). The remaining 17 patches had already been mown at the time of data collection so that we could only record the occurrence of *C. canaliculata* on those patches, while we could not analyze host plant size and density and did collect no egg clutches for determination of parasitism. The parameters obtained from the landscape model (described below) were also not available for all 77 patches. Therefore, the number of patches which we included in the analyses depended on the parameters used and is mentioned separately with each model.

3.2.2 Habitat quality

As parameters of habitat quality we measured for *C. canaliculata*: (1) mean host plant (*S. pratensis*) size, i.e. a factor derived via factor analysis from the parameters rosette

Table 3.1: Eigenvalues of the factors *Salvia* size and exposure obtained by principal components analysis. The factor *Salvia* size was derived from the three host plant parameters rosette diameter (cm), rosette height (cm), and number of vegetative cones. The factor exposure results from the two parameters potential solar irradiation (kWh/m^2) and exposure (°) of a patch.

Factor	**Included Parameters**	**PC 1**	**PC 2**	**PC 3**
Salvia size	Rosette diameter (cm)	-0.694	0.069	-0.717
	Rosette height (cm)	-0.651	0.366	0.665
	Number of vegetative cones	-0.308	-0.928	0.209
Exposure	Potential solar irradiation (kWh/m^2)	0.707	-0.707	–
	Exposure (°)	-0.707	-0.707	–

diameter (cm), rosette height (cm), and number of vegetative cones, (2) host plant density (m^2), (3) patch exposure, which was derived from the parameters exposure (in degrees, i.e. 0°/360° = north, 180° = south; cosine-transformed) and potential solar irradiation (kWh/m^2), and (4) patch slope (°). Table 3.1 shows the eigenvalues of the two factors. For obtaining parameters (1) and (2), a circle (r = 11 m) was created around the random point (see above) within each patch. Inside this circle, 10 squares of 1 m^2 size were randomly distributed. Within these squares, we counted all host plants and measured the rosette diameter, rosette height, and number of vegetative cones of 30 randomly selected host plants (three per square). We obtained parameters (3) and (4) from a digital landscape model, which was established for the Hohe Wann nature reserve during the MOSAIK-project (Schröder *et al.*, 2004; Rudner *et al.*, in press; Schröder *et al.*, submitted).

Regarding parasitism by *F. reptans*, we additionally counted the number of *C. canaliculata* egg clutches on the 30 above-mentioned plants per site and we took 10 egg clutches per site (if available) to the laboratory for determination of parasitism. Habitat quality parameters, which we analyzed for *F. reptans*, were (1) egg clutch density of *C. canaliculata*, (2) density of *S. pratensis* plants, (3) patch exposure, and (4) patch slope.

3.2.3 Patch size

We derived patch size (ha) from aerial photographs by means of GIS analysis. In addition, we used the radius of gyration (m), R, which is defined as the mean distance between the center of each grid cell (x_i, y_i) within a patch (n = number of grid cells) and the patch center (x, y) (equation 3.1, after Keitt *et al.*, 1997), to integrate patch shape into a further measure of patch size.

$$R = \frac{1}{n}\sqrt{(x_i - \bar{x})^2 + (y_i - \bar{y})^2} \tag{3.1}$$

We calculated the radius of gyration using the software Fragstats 3.3 (McGarigal, 2001).

3.2.4 Patch isolation

We used two different patch isolation metrics: (1) the shortest edge-to-edge distance (m) to the 'nearest neighbor' patch with *C. canaliculata* occurrence; (2) the connectivity S_i after Hanski (1998; equation 3.2), where P_j is the occurrence of *C. canaliculata* in patch j, A_j is the size (ha) of patch j, d_{ij} is the shortest center to center distance (m) of patches i and j (which are assumed to be circular), and $\alpha = 1/2D$, where D is the mean dispersal distance (m) of *C. canaliculata*.

$$S_i = \sum_{j=1}^{n} P_j \, e^{(-\alpha \, d_{ij})} \, A_j \tag{3.2}$$

The mean dispersal distance is unknown for *C. canaliculata*, but we estimated it to be 50 m, since we observed the beetle species so far only to move by walking (S. Reichmann and A. Heisswolf, personal observations). For *F. reptans*, which has a body length of only 1 mm and is probably not capable of active flight, we estimated the mean dispersal distance to be 10 m. We obtained parameter (1) using the GIS and we calculated parameter (2) using the software Isolator 1.3 (R. Biedermann, personal communication).

3.2.5 Statistics

We calculated all statistical procedures using the software package R 2.2.1 (R Development Core Team, 2005). Prior to analysis, we tested all parameters for correlation using Spearman rank-correlations. We included only those parameters into multiple models that were not correlated ($r_s < 0.5$). We combined correlated parameters to factors using principal components analysis (Mardia *et al.*, 1979; Venables & Ripley, 2002).

We tested the occurrence of *C. canaliculata* or *F. reptans* within a patch with logistic regression models, using the 'Design' package (Harrell, 2005). First, we calculated univariate models to pre-select parameters for multiple models. Then, we analyzed all possible combinations of the selected parameters. In order to evaluate the goodness-of-fit of the regression model we considered the coefficient of determination R^2 after Nagelkerke (1991) ($R^2_{Nagelkerke}$). We used ROC plots (receiver operating characteristics), i.e. the area under the resulting curve ('area under curve' = AUC), to determine the classification accuracy of the model (Hanley & McNeil, 1982; Zweig & Campbell, 1993). We further corrected the $R^2_{Nagelkerke}$- and AUC-values of models with multiple explanatory variables via bootstrapping, also using the 'Design' package (Harrell, 2005).

We tested the correlations between all parameters and population density of *C. canaliculata* or rate of parasitism by *F. reptans* using linear regression models. Again, we pre-selected parameters for multiple models based on univariate regressions. To evaluate the goodness-of-fit of the models and to compare multiple models, we used the adjusted coefficient of determination R^2.

For all analyses, we compared different models with the same response variable via the Akaike Information Criterion (*AIC*, see also Buckland *et al.*, 1997; Johnson & Omland, 2004), which allows choosing the model with the optimal compromise between goodness of fit and the lowest number of parameters. Additionally, we used hierarchical partitioning (Mac Nally, 2000, 2002; Heikkinen *et al.*, 2005) to determine the independent explanatory power of the predictor variables.

3.3 RESULTS

3.3.1 Incidence of C. canaliculata

We found egg clutches of *C. canaliculata* on 47 of 77 potential habitat patches (with occurrence of the only host plant *S. pratensis*), i.e. the prevalence of *C. canaliculata* was 61 %. In univariate regression models, which we used for parameter selection for the multiple models, the parameters slope, patch size, radius of gyration, and Hanski S correlated positively to the occurrence of *C. canaliculata*, the distance to the nearest neighbor negatively (Table 3.2).

Multiple logistic regression models resulted in 11 significant models (Table 3.3).

Table 3.2: Univariate logistic regression models explaining the occurrence of *C. canaliculata* depending on parameters of habitat quality (*Salvia* size [factor], *Salvia* density, exposure [factor], slope), habitat size (patch size, radius of gyration), and habitat isolation (distance to nearest neighbor = NN, Hanski S). Given are the intercept (± SE), regression coefficient (± SE), *P*-value, $R^2_{Nagelkerke}$, and *AUC*-value (± 95 % CI), as well as the number (*n*) of study sites that were included in the analysis. The parameters *Salvia* size and exposure are factors obtained by principal components analysis (cf. Material and Methods and Table 3.1).

Parameter	**Intercept ± SE**	**Coefficient ± SE**	*P*	R^2_N	*AUC*± **95 % CI**	*n*
Salvia size [factor]	0.406 ± 0.264	-0.031 ± 0.201	0.878	0.001	0.486 ± 0.151	60
Salvia density	0.451 ± 0.426	-0.008 ± 0.058	0.891	0	0.486 ± 0.156	60
Exposure [factor]	0.528 ± 0.264	0.287 ± 0.189	0.125	0.049	0.594 ± 0.154	64
Slope (°)	-0.997 ± 0.824	0.127 ± 0.067	0.049	0.080	0.651 ± 0.137	64
Patch size (ha)	-0.247 ± 0.344	0.047 ± 0.021	0.002	0.154	0.652 ± 0.124	77
Radius of gyration (m)	-0.545 ± 0.473	0.017 ± 0.008	0.010	0.111	0.660 ± 0.122	77
NN (m)	1.049 ± 0.356	-0.004 ± 0.002	0.014	0.103	0.629 ± 0.128	77
Hanski S	-0.053 ± 0.317	0.247 ± 0.119	0.015	0.100	0.677 ± 0.125	77

Table 3.3: Significant ($P < 0.01$, *AUC*-value ≥ 0.7) multivariate logistic regression models of the occurrence of *C. canaliculata* depending on combinations of parameters of habitat quality (slope), habitat size (patch size, radius of gyration), and habitat isolation (distance to nearest neighbor = NN, Hanski S). Given are the $R^2_{Nagelkerke}$, *AIC*-, and *AUC*-value (after internal validation via bootstrapping), as well as the number (n) of study sites that were included in the analysis. The model with the lowest *AIC* is printed in bold letters.

Parameter	R^2_N	*AIC*	*AUC*	*n*
Slope + patch size	0.158	80.27	0.695	64
Slope + NN	0.205	77.67	0.707	64
Slope + Hanski S	0.150	80.07	0.720	64
Patch size + NN	0.212	93.84	0.726	77
Patch size + Hanski S	0.228	92.19	0.748	77
Radius of gyration + NN	0.162	96.26	0.725	77
Radius of gyration + Hanski S	0.176	94.65	0.738	77
Slope + patch size + NN	**0.315**	**71.99**	**0.767**	**64**
Slope + patch size + Hanski S	0.262	73.81	0.775	64
Slope + radius of gyration + NN	0.264	74.30	0.743	64
Slope + radius of gyration + Hanski S	0.205	76.43	0.747	64

The model with the lowest *AIC*-value consisted of the parameters slope, patch size, and distance to the nearest neighbor (intercept ± SE = -1.08 ± 1.05, coefficient [patch slope] ± SE = 0.148 ± 0.080, coefficient [patch size] ± SE = 0.060 ± 0.029, coefficient [NN] ± SE = -0.007 ± 0.003, $R^2_{Nagelkerke} = 0.315$, $AUC = 0.767$, $AIC = 71.99$, $n = 64$).The predicted probability of occurrence calculated by this model and the observed occurrence of C. canaliculata for all studied habitat patches match very well for the majority of all studied patches (Figure 3.1). Furthermore, response surface plots show the predicted occurrence probability for three models considering each bivariate combination of the three model predictors patch slope, patch size, and distance to the nearest neighbor (Figure 3.2). The occurrence probability of *C. canaliculata* increased with increasing patch slope and size, as well as with decreasing distance to the nearest neighbor patch. Likewise, hierarchical partitioning showed that patch isolation (nearest neighbor or Hanski S), size, and slope had the highest independent explanatory power for *C. canaliculata* occurrence (nearest neighbor: 24 %, Hanski S: 22 %, patch size: 21 %, patch slope: 19 %), while the other tested parameters seem to be of little relevance (Figure 3.3).

3.3.2 Population density of C. canaliculata

The parameters *Salvia* density, patch size, and radius of gyration correlated positively with population (i.e. egg clutch) density of *C. canaliculata* in univariate linear regression models (Table 3.4). Both parameter combinations resulted in significant multiple models (Table 3.5), however, an increasing *Salvia* density and patch size explained

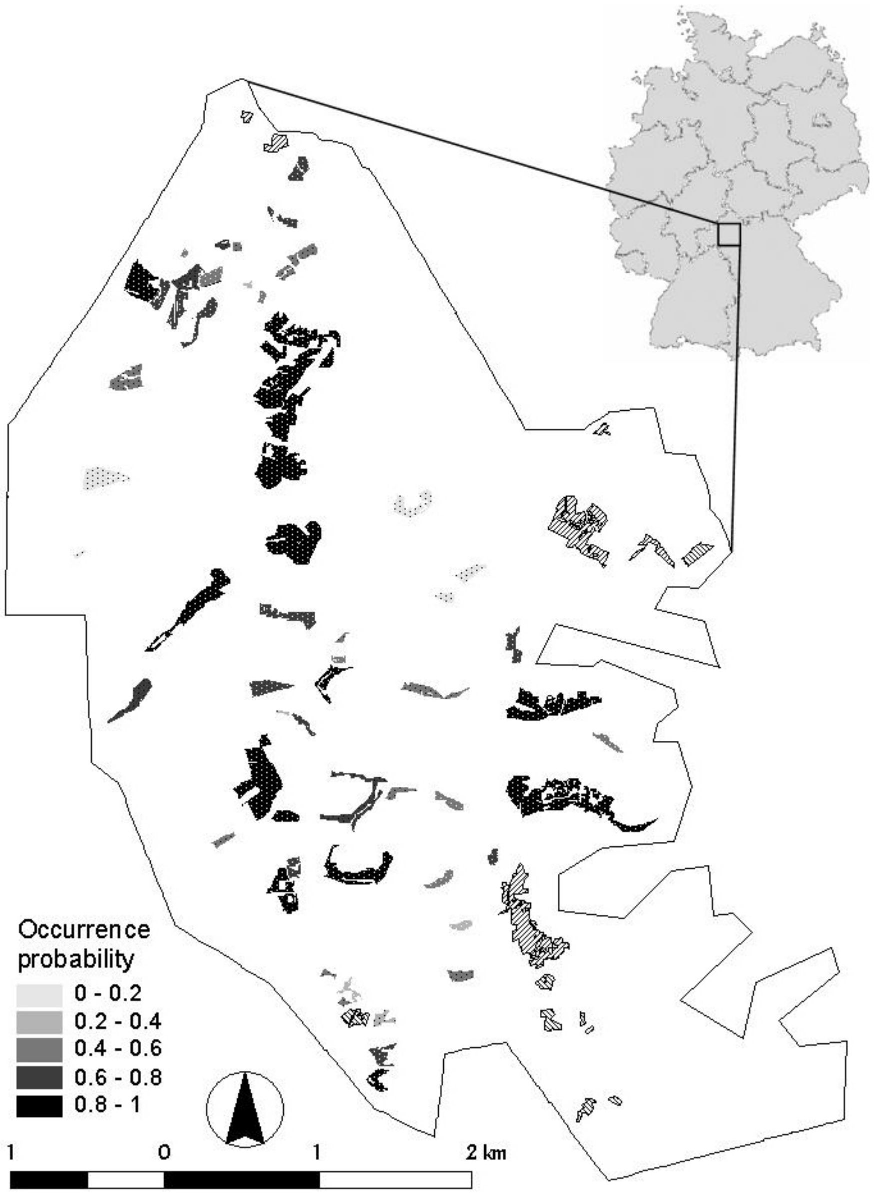

Figure 3.1: Predicted and observed occurrence of *C. canaliculata* within the Hohe Wann nature reserve. The map shows the distribution of the 77 studied potential habitat patches with occurrence of the host plant *S. pratensis*. Additionally, a small map of Germany shows the approximate location of the nature reserve within Germany. The studied patches are colored in different shades of gray corresponding to the occurrence probabilities of *C. canaliculata* predicted by the multiple logistic regression model depending on the parameters patch slope, patch size, and distance to the nearest neighbor. White (presence) and black (absence) dots on the respective patches indicate the observed occurrence of *C. canaliculata*. Patches on which the presence or absence of *C. canaliculata* was registered, but which could not be included into the multiple model due to the lack of area-wide data are not shaded but are left-hatched (presence) or right-hatched (absence).

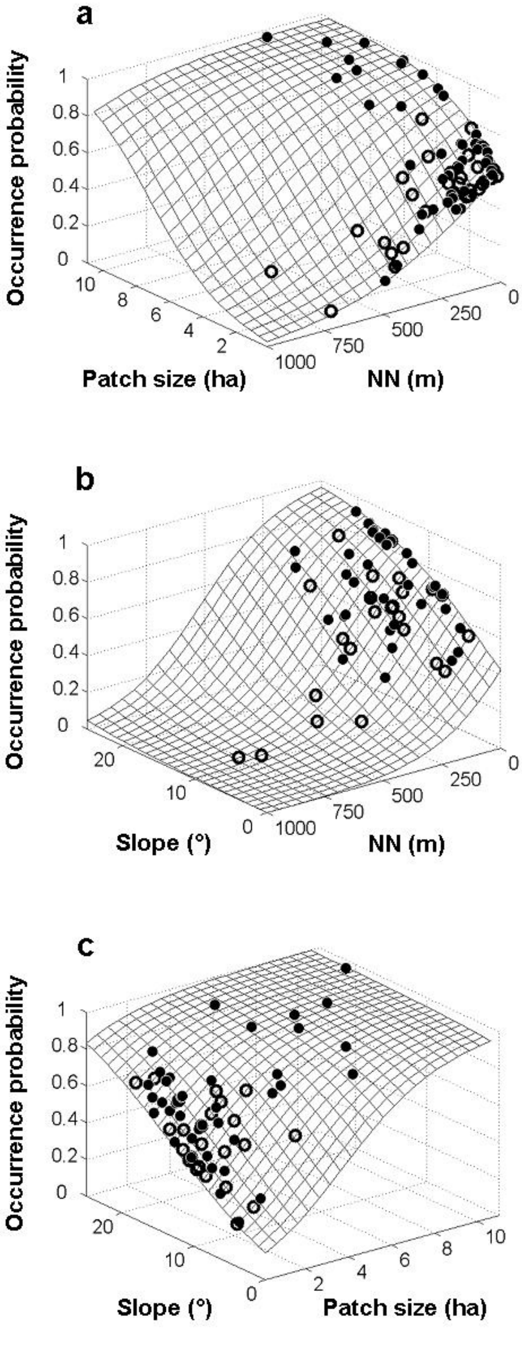

Figure 3.2: Bivariate habitat suitability models for *C. canaliculata*. The grid surfaces indicate the estimated models for the parameter combinations (a) patch size (ha) and distance to the nearest neighbor = NN (m), (b) patch slope (°) and patch size (ha), and (c) patch slope (°) and distance to the nearest neighbor = NN (m). The field data, i.e. observed presence and absence of the species, are represented by filled and empty circles. The occurrence probability of *C. canaliculata* increases with increasing patch size and patch slope, as well as with decreasing distance to the nearest neighbor.

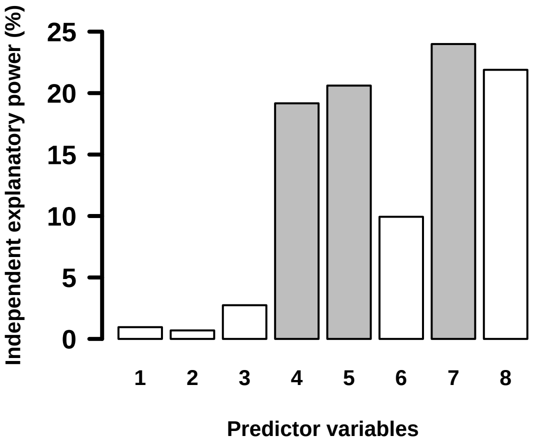

Figure 3.3: Parameters explaining the occurrence of *C. canaliculata*. Percentage distribution of independent explanatory power of all tested predictor variables calculated by hierarchical partitioning. The tested variables were (1) *Salvia* size [factor], (2) *Salvia* density, (3) patch exposure [factor], (4) patch slope, (5) patch size, (6) radius of gyration, (7) distance to the nearest neighbor, and (8) Hanski S. Parameters that were included in the best multiple model are shaded in gray.

best the increase in population density of *C. canaliculata*. Additionally, hierarchical partitioning showed that *Salvia* density had an almost three times higher independent explanatory power (i.e. 54 %) for the population density of *C. canaliculata* than patch size (19 %, Figure 3.4).

Table 3.4: Univariate linear regression models explaining the population density of *C. canaliculata* depending on parameters of habitat quality (*Salvia* size [factor], *Salvia* density, exposure [factor], slope), habitat size (patch size, radius of gyration), and habitat isolation (distance to nearest neighbor = NN, Hanski S). Given are the intercept (± SE), regression coefficient (± SE), *P*-value, and adjusted R^2-value, as well as the number (*n*) of study sites that were included in the analysis. The parameters *Salvia* size and exposure are factors obtained by principal components analysis (cf. Material and Methods and Table 3.1).

Parameter	**Intercept ± SE**	**Coefficient ± SE**	*P*	R^2	*n*
Salvia size [factor]	0.316 ± 0.066	0.046 ± 0.050	0.360	-0.003	60
Salvia density	0.098 ± 0.101	0.038 ± 0.014	0.008	0.100	60
Exposure [factor]	0.311 ± 0.068	0.026 ± 0.053	0.628	-0.016	49
Slope (°)	0.407 ± 0.203	-0.008 ± 0.016	0.632	-0.016	49
Patch size (ha)	0.126 ± 0.079	0.009 ± 0.002	0.001	0.176	60
Radius of gyration (m)	0.043 ± 0.112	0.004 ± 0.001	0.005	0.113	60
NN (m)	0.416 ± 0.093	-0.001 ± 3.78e-04	0.134	0.022	60
Hanski S	0.273 ± 0.082	0.021 ± 0.024	0.380	-0.004	60

Table 3.5: Significant ($P < 0.001$) multivariate linear regression models explaining the population density of *C. canaliculata* depending on combinations of parameters of habitat quality (*Salvia* density) and habitat size (patch size, radius of gyration). Given are the intercept (± SE), regression coefficients 1 and 2 (± SE), adjusted R^2-value, and *AIC*-value, as well as the number (*n*) of study sites that were included in the analysis. The best model is printed in bold letters.

Parameter	Intercept ± SE	Coeff 1 ± SE	Coeff 2 ± SE	R^2	*AIC*	*n*
***Salvia* density** **+ patch size**	**-0.140 ± 0.103**	**0.043 ± 0.012**	**0.010 ± 0.002**	**0.316**	**71.52**	**60**
Salvia density + radius of gyration	-0.219 ± 0.131	0.041 ± 0.013	0.004 ± 0.001	0.240	77.87	60

3.3.3 Parasitism by F. reptans

We observed parasitism of *C. canaliculata* egg clutches by the hymenopteran wasp *F. reptans* on 17 of the 31 patches (i.e. 55 %) where *C. canaliculata* occurred and on which 10 egg clutches could be collected. The only parameter that significantly explained the occurrence of *F. reptans* was the egg clutch density of *C. canaliculata*, i.e. the probability of parasitism was positively density dependent (Table 3.6). None of the other studied parameters correlated significantly with the parasitism rate of *F. reptans* (Table 3.7).

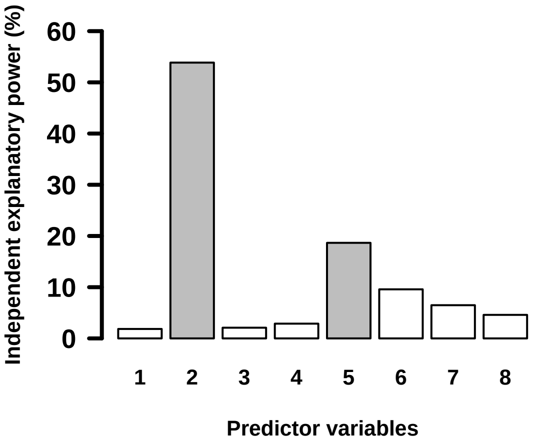

Figure 3.4: Parameters explaining the population density of *C. canaliculata*. Percentage distribution of independent explanatory power of all tested predictor variables calculated by hierarchical partitioning. The tested variables were (1) *Salvia* size [factor], (2) *Salvia* density, (3) patch exposure [factor], (4) patch slope, (5) patch size, (6) radius of gyration, (7) distance to the nearest neighbor, and (8) Hanski S. Parameters that were included in the best multiple model are shaded in gray.

Table 3.6: Univariate logistic regression models explaining the occurrence of *F. reptans* depending on parameters of habitat quality (egg clutch density of *C. canaliculata*, *Salvia* density, exposure [factor], slope), habitat size (patch size, radius of gyration), and habitat isolation (distance to nearest neighbor = NN, Hanski S). Given are the intercept (± SE), regression coefficient (± SE), *P*-value, $R^2_{Nagelkerke}$, and *AUC*-value (± 95 % CI), as well as the number (*n*) of study sites that were included in the analysis. The parameter exposure is a factor obtained by principal components analysis (cf. Material and Methods and Table 3.1). The only parameter, which significantly influenced the occurrence of *F. reptans* is printed in bold letters.

Parameter	**Intercept ± SE**	**Coefficient ± SE**	*P*	R^2_N	*AUC*± **95 % CI**	*n*
Egg clutch density	**-0.778 ± 0.588**	**1.804 ± 0.947**	**0.022**	**0.207**	**0.788 ± 0.186**	**31**
Salvia density	-0.157 ± 0.624	0.059 ± 0.087	0.482	0.021	0.668 ± 0.218	31
Exposure [factor]	0.228 ± 0.393	0.255 ± 0.293	0.376	0.038	0.578 ± 0.224	27
Slope (°)	0.158 ± 1.295	0.005 ± 0.095	0.958	0	0.503 ± 0.251	27
Patch size (ha)	-0.133 ± 0.525	0.011 ± 0.013	0.390	0.031	0.631 ± 0.211	31
Radius of gyration (m)	-0.658 ± 0.743	0.010 ± 0.008	0.178	0.076	0.605 ± 0.207	31
NN (m)	-0.272 ± 0.578	0.002 ± 0.002	0.277	0.050	0.567 ± 0.217	31
Hanski S	0.309 ± 0.377	-1.018 ± 1.445	0.222	0.063	0.582 ± 0.207	31

Table 3.7: Univariate linear regression models explaining the parasitism rate of *F. reptans* depending on parameters of habitat quality (egg clutch density of *C. canaliculata*, *Salvia* density, exposure [factor], slope), habitat size (patch size± SE), regression coefficient (± SE), *P*-value, and adjusted R^2-value, as well as the number (*n*) of study sites that were included in the analysis. The parameter exposure is a factor obtained by principal components analysis (cf. Material and Methods and Table 3.1).

Parameter	**Intercept ± SE**	**Coefficient ± SE**	*P*	R^2	*n*
Egg clutch density	0.294 ± 0.098	0.076 ± 0.106	0.479	-0.020	31
Salvia density	0.429 ± 0.110	-0.012 ± 0.014	0.398	-0.011	31
Exposure [factor]	0.334 ± 0.066	0.046 ± 0.053	0.393	-0.011	27
Slope (°)	0.177 ± 0.222	0.013 ± 0.017	0.437	-0.017	27
Patch size (ha)	0.326 ± 0.090	0.001 ± 0.002	0.733	-0.038	31
Radius of gyration (m)	0.201 ± 0.116	0.002 ± 0.001	0.154	0.047	31
NN (m)	0.305 ± 0.087	2.04e-04 ± 2.89e-04	0.487	-0.021	31
Hanski S	0.367 ± 0.060	-0.096 ± 0.078	0.229	0.022	31

3.4 DISCUSSION

Habitat fragmentation due to anthropogenic land use is a serious problem for the persistence of both rare habitat types and endangered species that are restricted to these habitats (Kruess & Tscharntke, 1994; Fahrig, 2001; Tscharntke *et al.*, 2002; Tscharntke & Brandl, 2004). In the present study, we analyzed the influence of patch size, isolation, and quality on occurrence and population density of the endangered specialized leaf beetle *Cassida canaliculata*. Additionally, we investigated the influence of these parameters and of the population density of *C. canaliculata* on the occurrence and rate of parasitism of the egg parasitoid *Foersterella reptans*.

Regarding the question, which of the factors patch size, isolation, and quality is most important for the occurrence of a species within a potential habitat patch, controversial results can be found in the literature (Thomas, 1984; Dennis & Eales, 1997; Hanski & Singer, 2001; Tscharntke *et al.*, 2002; Hanski & Gaggiotti, 2004; Krauss *et al.*, 2005). Classical metapopulation theory (Hanski & Gaggiotti, 2004) focuses on the importance of patch size and isolation for the occurrence of a species within a patch, i.e. it predicts that the occurrence probability will increase with increasing patch size and with decreasing isolation. However, several studies were able to show that habitat quality can be as important for species occurrence as the classical metapopulation parameters (e.g. Clarke *et al.*, 1997; Dennis & Eales, 1997; Thomas *et al.*, 1998; Wiegand *et al.*, 1999). Consequently, Thomas *et al.* (2001) suggested that a combination of all three factors might be important for enhancing the survival of a species within a fragmented landscape. While the size and isolation of a patch can influence the (re-)colonization probability of a patch, habitat quality may be crucial for the survival probability of a population within a patch by increasing the carrying capacity of the patch.

In accordance with this theory, the occurrence probability of *C. canaliculata* correlated positively with patch size, negatively with patch isolation, and positively with the slope of a patch, which was one of the habitat quality parameters. As already mentioned above, patch size can be important for species occurrence, because dispersing animals may more easily find larger patches and larger patches also can support larger populations. Additionally, patch shape may be essential, as edge effects at the patch border can have a negative impact on species survival within a patch (Saunders *et al.*, 1991; Tscharntke *et al.*, 2002). Thus, compact shaped patches with a small border length to patch size ratio may have a higher occurrence probability. The results of the present study, however, suggest that patch shape, which we measured as radius of gyration, is less important for the occurrence of *C. canaliculata* than patch size. According to hierarchical partitioning, patch size had a two times higher independent explanatory power (21 %) for the occurrence of the leaf beetle on a patch than the radius of gyration (10 %), although the correlation between both predictors was quite high ($r_s = 0.96$). Therefore, in this study, linear landscape elements (with a comparably high radius of gyration and low patch size) do not play an important role for explaining the distribution of *C. canaliculata*.

Concerning patch isolation, the parameters distance to the nearest neighbor and connectivity (after Hanski, 1998) describe the isolation of a patch qualitatively different. The distance to the nearest neighbor patch with *C. canaliculata* occurrence gives no information on the size of this patch and on how many other patches are located nearby, whereas the connectivity integrates the size and distance of all potential source patches within a landscape (cf. Kuhn & Kleyer, 1999). In a recent meta-analysis, Moilanen & Nieminen (2002) recommended that complex connectivity measures should be preferred over simple measures (like the distance to the nearest neighbor), as simple measures often fail to detect effects of isolation, which could be detected with measures that are more complex. Likewise, in the present study, using connectivity as a predictor of *C. canaliculata* occurrence resulted in the highest *AUC*-value of all univariate models. However, in all multiple models, the distance to the nearest neighbor patch had a stronger influence on the occurrence probability of *C. canaliculata* than the connectivity. Likewise, the independent explanatory power was a little higher for the nearest neighbor (24 %) than for the connectivity (22 %).

One possible explanation for these results may be that – in contrast to Moilanen & Nieminen (2002), who focused their analysis on butterfly species – the shortest distance to an occupied patch may be more relevant to a relatively immobile species like *C. canaliculata* (A. Heisswolf, personal observation) than a high ‘global’ connectivity. An advantage of the nearest neighbor measure could also be that it was calculated as edge-to-edge distance, which is probably more relevant for the dispersing animal, while the connectivity after Hanski used center-to-center distances and assumed all patches circular. A third reason for the better performance of the more simple isolation measure may be that the calculation of the connectivity requires an estimation of the mean dispersal distance of the species. This distance is unknown for *C. canaliculata* and we estimated it to be 50 m, which may have not been adequate. However, we repeated the connectivity analyses with several different mean dispersal distances between 10 and 100 m (data not shown). In these analyses, a distance of 50 m resulted in the best model fit, which implies that our estimation was rather tolerable.

In conclusion, both isolation measures can be used to explain the occurrence of *C. canaliculata* within a patch, however, as the distance to the nearest occupied neighbor patch requires no information on the dispersal ability of *C. canaliculata* it should be preferred over the more complex connectivity measure. Furthermore, a still better measure of patch isolation from the beetles’ view should also include the structure of the ‘matrix’ into which the potential habitat patches are embedded (Gustafson & Gardner, 1996; Ricketts, 2001; Goodwin & Fahrig, 2002). Habitat patches that have the same distances to the ‘nearest neighbor’ may be differently isolated depending on the surrounding matrix structure. However, as we have no detailed information on the dispersal abilities of *C. canaliculata* depending on matrix type, the matrix was not included in the analysis of patch isolation in the present study.

Regarding habitat quality, only patch slope contributed significantly to the occurrence probability of the beetle. We cannot explain the influence of this parameter as straightforward as the influence of habitat size and isolation. A possible explanation could be that the slope of a patch usually correlates with the microclimate of the patch, i.e. steeper patches often have a warmer microclimate, which *C. canaliculata* may prefer. In agreement with this hypothesis, we found a positive relationship between both patch exposition and microclimate and the occurrence of *C. canaliculata* in a previous study (Heisswolf *et al.*, in press). However, we could find no such correlations in the present study.

Another possible explanation could be that steep patches are less often disturbed by management than less steep patches and may thus provide better conditions for the long-term survival of populations. As we have no detailed information on management intensity in relation to patch slope this hypothesis remains speculative. Thus, we cannot finally elucidate the meaning of the slope of a patch for the occurrence of *C. canaliculata* within the scope of this study. Host plant density, which had an effect on beetle occurrence in the above-mentioned previous study (Heisswolf *et al.*, in press) as well as e.g. in the endangered butterfly *Polyommatus coridon* (Krauss *et al.*, 2005), did not influence the occurrence of *C. canaliculata* in the present study.

While the influence of habitat quality on *C. canaliculata* occurrence remains to be elucidated in more detail, there was a clear correlation between habitat quality and population density of *C. canaliculata*. Egg clutch density of *C. canaliculata*, which we used as a measure of population density within a patch, increased not only with patch size but also with host plant density, i.e. large habitats with high host plant densities supported larger populations of *C. canaliculata*. In contrast to other studies (Bach, 1988; Connor *et al.*, 2000; Krauss *et al.*, 2004, 2005) patch size and host plant density were not correlated ($r_s = -0.008$) in the study area. Consequently, viable populations of *C. canaliculata* may also persist on smaller patches with high host plant densities (cf. Thomas *et al.*, 2001). Therefore, it may not only be important to protect large habitats but also to maintain smaller-sized patches with high host plant densities to facilitate the survival of *C. canaliculata* within a fragmented landscape.

Regarding the egg parasitoid, only the egg clutch density of *C. canaliculata* affected the occurrence of *Foersterella reptans*, i.e. the probability of parasitism was positively density dependent. None of the other studied parameters correlated significantly with either the occurrence or density of *F. reptans*. One possible reason for this lack of correlation may be that *F. reptans* also parasitizes other *Cassida* species (e.g. *C. rubiginosa* Müller), and therefore parameters of habitat quality, size, and isolation – which were appropriate with regard to its host *C. canaliculata* – may have not been comprehensive enough for the perspective of the parasitoid. For example, *C. rubiginosa* lives on thistles (Asteraceae: Carduae), which only rarely grow in the same habitat as *S. pratensis*, the host plant of *C. canaliculata*. Thus, *C. canaliculata* patches may not have been the only suitable patches for *F. reptans*. Moreover, even if

F. reptans would parasitize only *C. canaliculata* the spatial scales affecting herbivore and parasitoid may be nevertheless different (Tscharntke & Brandl, 2004; Cronin & Reeve, 2005; Heisswolf *et al.*, in press). Therefore, the parasitoid may perceive both patch size and isolation quite differently. Only detailed studies on the distribution of all other potential hosts and on the dispersal abilities of *F. reptans* may lead to a better estimation of these parameters for the parasitoid. However, this was far beyond the scope of this study.

In summary, our study corroborates the hypothesis of e.g. Dennis & Eales (1997) and Thomas *et al.* (2001) that habitat quality is as important for the survival of insect species in fragmented landscapes as patch size and isolation. While all three, patch quality, size, and isolation determined the occurrence of *C. canaliculata*, population density was affected most by habitat quality. Thus, we recommend to preserve not only large habitat patches, but also to maintain large host plant densities in all potential habitat patches to enhance the survival of *C. canaliculata* within the Hohe Wann nature reserve.

Chapter 4

Olfactory *versus* contact cues in host plant recognition of a monophagous chrysomelid beetle

with Dirk Gabler, Elisabeth Obermaier and Caroline Müller

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Abstract. The importance of olfactory versus contact cues in the process of host plant recognition was investigated in the tortoise beetle *Cassida canaliculata* (Coleoptera: Chrysomelidae), which is strictly monophagous on meadow sage (*Salvia pratensis*). The reaction of adult beetles to olfactory host cues was tested using three olfactory bioassays: a locomotion compensator, a six-chamber-olfactometer, and a 'stem arena' without contact. The importance of contact cues was studied using a 'stem arena' with contact and a bioassay-guided fractionation of plant extracts was elaborated to characterize the nature of contact stimuli. We conclude that olfactory cues play only a weak role in the host recognition process of *C. canaliculata* whereas at least two distinct non-polar contact stimuli acting in concert are sufficient for host plant identification.

4.1 INTRODUCTION

For herbivorous insects, a natural habitat represents a highly diverse mosaic of plants in which they must recognize cues that are emitted by their potential hosts. Some insects may encounter plants simply by chance, after which contact chemoreception allows them to distinguish between potential hosts and non-hosts (Jermy *et al.*, 1988; Chapman & Sword, 1993; Mitchell, 1994; Schoonhoven *et al.*, 1998). However, others also appear to be able to perceive and use olfactory and visual cues at a distance from a plant (Feeny *et al.*, 1970; Andersen & Metcalf, 1986; Visser, 1986; Mitchell, 1994; Cook & Neal, 1999; Müller & Hilker, 2000).

Vision plays a role in host plant location of some herbivorous beetles (Tanton, 1977; Hausmann *et al.*, 2004), but the importance of visual stimuli for host plant recognition still needs to be analyzed in the majority of species. Recently, it has been demonstrated that certain herbivores, as well as carnivores, can be very sensitive to spectral composition of (herbivore-infested) leaves (Bullas-Appleton *et al.*, 2004; Fischer *et al.*, 2004; Mäntylä *et al.*, 2004). Plant size and shape are further optical features, which insects could use for host plant identification. However, within a given plant species, there may be large morphological variation among plant individuals within a site, from site to site, as well as from season to season (Prokopy & Owens, 1983). Thus, plant shape and size might be less reliable cues for host identification. Moreover, dense vegetation can hamper visual host plant detection from a distance (Rausher, 1981), especially in beetles that walk rather than fly.

Olfactory cues are likely to be signals that are more reliable for host plant recognition. They may allow for host plant location even in a complex environment, if the insect central nervous system receives the volatile information at a fine-scale spatio-temporal resolution (Held *et al.*, 2003; Bruce *et al.*, 2005). Many beetle species are indeed able to identify their host plant based on olfactory cues (e.g. Bartlet *et al.*, 1997; Kalberer *et al.*, 2001; Zhang & Schlyter, 2004; Kalberer *et al.*, 2005). Such olfactory cues could be species-specific compounds (Feeny *et al.*, 1970; Blight *et al.*, 1995; Bartlet *et al.*, 1997) or specific ratios of ubiquitous compounds (Visser & Avé, 1978; Barata *et al.*, 2000; van Tol & Visser, 2002), the latter scenario being in general more common (Bruce *et al.*, 2005).

The most accurate and reliable information about host suitability is gained via contact chemoreception. Typical behaviors before acceptance or rejection of a host are antennating, palpating, test biting, and test feeding (Harrison, 1987). On the plant surface, plant cuticular waxes can already give important information for host plant acceptance (Eigenbrode & Espelie, 1995; Müller & Riederer, 2005). After test-biting, individual host-specific compounds can be sufficient to stimulate feeding in several specialist herbivores. The monophagous beetle *Ceutorhynchus inaffectatus* Gyllenhal (Coleoptera: Curculionidae) is stimulated by allyl-glucosinolate of its host plant, a Brassicaceae (Larsen *et al.*, 1992), while cucurbitacin can be sufficient to stimulate feeding of some specialists on Cucurbitaceae (Metcalf *et al.*, 1980; Tallamy & Krischik, 1989). However, in other species feeding is stimulated only by a mixture of several compounds (Müller & Renwick, 2001; van Loon *et al.*, 2002), which may be inactive when offered individually, but show synergistic effects when offered together (Endo *et al.*, 2004; Tamura *et al.*, 2004).

The specialized tortoise beetle *Cassida canaliculata* Laich. (Coleoptera: Chrysomelidae) is strictly monophagous on meadow sage (*Salvia pratensis* L., Lamiales: Lamiaceae) (Wencker & Silbermann, 1866; Bourgeois & Scherdlin, 1899; Reitter, 1912; Graser, 1984; Trautner *et al.*, 1989; A. Heisswolf and D. Gabler, unpublished data). Thus, the beetles should be very specifically able to recognize their host plant species. While little is published on the ecology of *C. canaliculata* (Steinhausen, 1949; Trautner *et al.*, 1989; Heisswolf *et al.*, 2005, in press), nothing is known about the host recognition process in this species. Within Germany, *C. canaliculata* is endangered, in Bavaria it is even threatened by extinction (Kippenberg, 2003); however, on warm slopes in the study area (Hohe Wann nature reserve in Northern Bavaria) it is locally highly abundant (A. Heisswolf and E. Obermaier, personal observations).

In this study, the attraction behavior of *C. canaliculata* to olfactory and contact cues of its host plant *S. pratensis* was analyzed in laboratory bioassays. Bioassay-guided fractionation of plant extracts was elaborated to characterize the nature of contact stimuli.

4.2 MATERIAL AND METHODS

4.2.1 Insects

Adults of *C. canaliculata* were collected in the Hohe Wann nature reserve in Northern Bavaria, Germany (50°03´N, 10°35´E), between May and July 2005. They were kept in boxes (200 x 200 x 90 mm) with a gauze lid (500 µm mesh) in a climatic chamber at 20 °C, 70 % relative humidity, and 16L:8D. The bottom of each box was covered with filter paper and the beetles were fed on leaves of *S. pratensis*.

4.2.2 Extracts of **Salvia pratensis**

Leaf material of *S. pratensis* was harvested from pre-flowering plants growing in the botanical garden of University of Würzburg, frozen, and lyophilized for 17 hrs. Dried leaves were crushed in a mortar with addition of sea sand (Merck) and extracted in

150 ml of either *n*-hexane, dichloromethane, or methanol (Carl Roth GmbH, Karlsruhe, Germany). The three resulting extracts of different polarity were filtered and concentrated in a rotary evaporator.

Assuming that the chemical quality of stems, petioles, and leaves is similar, both intact plant stems as well as stem dummies treated with leaf extracts were offered in the behavioral contact assays (see below for a detailed description). The volume of the extracts used in these assays corresponded to the average weight of a plant stem of 15 mm length and 5 mm diameter (like the stems used in the contact bioassays), which was 0.15 g. The corresponding volumes for the three extracts were 8 μl (*n*-hexane), 5 μl (dichloromethane), and 14 μl (methanol).

The *n*-hexane extract was further fractionated: 1 ml of the extract was evaporated to dryness and the residue dissolved in 200 μl of dichloromethane. This solution was loaded onto a column (Isolate SPE Columns 100 mg Si) washed with dichloromethane. Elution was carried out sequentially with the following five solvents: (1) 100 % *n*-hexane, (2) 90 % *n*-hexane + 10 % dichloromethane, (3) 50 % *n*-hexane + 50 % dichloromethane, (4) 90 % dichloromethane + 10 % methanol, and (5) 100 % methanol. For each elution step, 1 ml of the respective solvent was used and the resulting fractions were collected separately.

4.2.3 Olfactory bioassays: locomotion compensator

The reactions of beetles to olfactory plant cues were tested with three different assays. First, the behavioral response of walking beetles to volatiles from different sources borne in an air stream was tested on a locomotion compensator. In this assay, an individual beetle is maintained at the top of a servosphere (diameter: 300 mm; Tracksphere LC 300, Syntech, Hilversum, The Netherlands) to which the air stream is directed. Two motors compensate displacements of the moving animal and pulse generators monitor the resulting movement of the sphere (Kramer, 1976), which allows the reconstruction of the tracks described by the beetle. The following four track parameters were used to quantify the beetle's behavior: (1) walking speed (mm/s); (2) straightness of walking, i.e. the quotient of vector length and total track length (ranging from 0 to 1); (3) upwind length (mm), i.e. the net distance from the origin towards the odor source along a straight line; and (4) upwind fixation, the quotient of upwind length and total track length (ranging from -1 to +1).

The beetles were starved three to four hours prior to testing. Per treatment, each beetle was allowed to acclimatize on the sphere for one minute, and then one of five different odor sources was applied for four minutes. Tested odor sources were (1) five leaves (5-6 g) of *S. pratensis*, (2) potted, one-year-old pre-flowering *S. pratensis* plants with 8-10 leaves, (3) a pot containing only soil, (4) a pure air stream, and (5) no air stream at all. The latter three treatments served as controls to test for the beetle's general walking behavior. The odor sources were presented as follows: compressed air was passed through a charcoal filter and a flask filled with distilled water to obtain a moisturized clean airflow. The airflow was passed through a flow meter, which adjusted the flow to 1200 ml/min. Then the flow was passed through another flask, which contained either the materials described above or nothing for the pure air stream. The volume of the flask was 0.5 l (glass flask) for the empty control and the test with leaf material and 1.0 l (polyethylene flask) for the potted plant as well as for the pot containing only soil. Finally, the airflow ended up in the delivery tube (inner diameter: 15 mm), positioned with its mid-axis the same height as, but approximately 40 mm away from the top of the sphere.

4.2.4 Olfactory bioassays: six-chamber-olfactometer

In a second assay, a static six-chamber olfactometer corresponding to the four-chamber olfactometer described by Steidle & Schöller (1997) was used. The olfactometer was made of acrylic glass, consisting of a cylinder (40 mm high, diameter: 170 mm) divided by vertical plates into six equal chambers. On top of the cylinder, a removable walking arena (diameter: 160 mm) was placed, consisting of plastic gauze (210 μm mesh) with a rim of acrylic glass (15 mm high). The whole olfactometer was covered with a glass plate, and no airflow was generated.

Fresh leaf material (whole leaves) was placed in one of the chambers in either of two quantities: (1) one leaf (0.2-0.3 g; low quantity) and (2) six leaves (2.5-3.4 g, high quantity). The other five chambers remained empty and served as a control. To avoid biased results due to possible side preferences of the beetles, the position of the samples and the controls was rotated clockwise after every trial. After four trials, the leaf material was replaced. The experiments were performed in a dark room and a central light source above the olfactometer was used for illumination. Contamination of the walking arena with sample odors or possible pheromones of the beetles was avoided by cleaning the walking arenas and glass plates with ethanol and demineralized water between trials. As in nature the beetles usually walk on the lower side of plant leaves, an inverted setup was additionally tested in which the olfactometer was turned upside down and the movement of the beetles was followed by using a mirror. In this setup, three leaves (0.8-1.8 g, medium quantity) were offered.

In all settings, the beetles were starved at least two hours prior to testing. Then, each individually tested beetle was allowed to acclimatize in the arena for five minutes before the observation started. Using the software The Observer 5.0 (Noldus Information Technology, Wageningen, The Netherlands), the location (chamber) as well as the activity status (active: walking; inactive: resting, grooming) of each beetle was recorded for five minutes. The time the beetles spent walking above the chamber containing the leaf material was compared with the walking time on empty control chambers and used to assess the attractive effect of the host material.

4.2.5 Olfactory bioassays: stem arena without contact

In a third assay for testing the reaction of *C. canaliculata* to olfactory cues, a ‘stem arena’ (according to Müller & Hilker, 2000; Figure 4.1) was used, as beetles were observed to preferably climb objects. Stems of the host plant (height: 15 mm, diameter: 5 mm) as well as stem dummies (toothpicks) were enclosed with fine wire mesh cylinders (height: 20 mm, diameter: 15 mm, 1 mm mesh) to prevent contact. In the bottom of a petri dish (diameter: 55 mm), two enclosed stems and two enclosed dummies were offered to adult beetles in an alternating pattern. The bottom of the petri dish was filled with soil covered by filter paper to fix the stems and dummies. The stem arena was placed in the bottom of a larger petri dish (diameter: 90 mm) filled with water to prevent beetles from escaping.

In this arena without contact as well as in all other ‘stem arena’ assays described below, the beetles were starved at least two hours prior to testing. Then, one adult beetle was placed in the center of the arena in a supine position. The time the beetles spent on test or control stems was recorded continuously for 8 minutes using the software The Observer 5.0.

4.2.6 Contact bioassays: stem arena with contact

The ‘stem arena’ (Figure 4.1) was also used to test the role of contact cues in host recognition of *C. canaliculata*. In this assay, stems of the host *S. pratensis*, the non-host yarrow, *Achillea millefolium* L. (Asterales: Asteraceae), or stem dummies were offered to adult beetles in an alternating pattern, however, without any cover. The time the beetles spent on test or control stems was recorded continuously for 8-10 minutes.

First, it was tested whether *C. canaliculata* is able to discriminate between stems of its host plant and stems of yarrow (*Achillea millefolium*), a non-host plant that is very common in the natural habitat of *C. canaliculata*. Second, in order to account for a possible deterring effect of the non-host plant, stems of yarrow were offered against stem dummies (toothpicks). In the third experimental series, stem dummies, i.e. cigarette filters (height: 15 mm, diameter: 5 mm; ZIG-ZAG Slim Filters, Manchester, UK), treated with host plant extracts of different polarity (see above) were offered against stem dummies treated with the corresponding solvent only. In the fourth experimental series, the beetles’ response to the five different fractions, as well as 1:1 mixtures of specific fractions of the *n*-hexane extract (see above) were tested against the respective solvents.

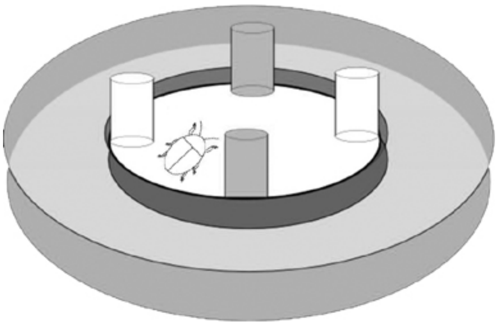

Figure 4.1: Stem arena used for bioassays with adults of *Cassida canaliculata*. *Gray* test stems; *white* control stems (each 15 mm high); a petri dish (diameter: 55 mm, height: 10 mm) was used as arena. Test and control stems were placed at the edge of the dish in an alternating pattern. The bottom of the arena was filled with soil and covered by a filter paper. The stem arena was placed in the bottom of a larger petri dish (diameter: 90 mm) filled with water to prevent beetles from escaping. Drawing by D. Gabler and C. Müller.

4.2.7 Statistics

All parameters were tested for normal distribution using the Shapiro–Wilk test. On the locomotion compensator, the parameters ‘straightness’ (ranging from 0 to 1) and ‘upwind straightness’ (ranging from -1 to +1) were arcsine-transformed prior to analysis. Walking parameters were compared between test odors and the respective controls using the Mann–Whitney U-test. Additionally, upwind length and upwind straightness were tested against zero using the Mann–Whitney U-test.

Differences in walking times above the six chambers in the olfactometer bioassay were compared using the Friedman test followed by the Bonferroni corrected Wilcoxon matched–pairs test. Additionally, the test chamber (containing the leaf material) was also compared to the control chamber directly opposite to the test chamber using the Wilcoxon signed–rank test for paired samples.

In the stem arena assays, the relative difference between the times spent on the test and control stem, i.e. (time on test stem – time on control stem) / total time on both stems was calculated for each beetle. The corresponding null hypothesis is that beetles have no preference, i.e. spend as much time on test as on control stems, and consequently that the relative difference between these two times is zero. The relative differences can only range from -1 (time spent completely on the control stem) to +1 (time spent completely on the test stem) and were thus arcsine-transformed prior to further analysis. The transformed differences were tested against the null hypothesis using the Mann–Whitney U-test. Beetles that were inactive for more than half of the observation time were excluded from all analyses as they were considered to be not motivated. All statistical procedures were calculated with the software package R 2.2.0 for Windows (R Development Core Team, 2005).

4.3 RESULTS

4.3.1 Response to volatile cues

On the locomotion compensator, neither of the recorded walking parameters of the beetles (walking speed, straightness, upwind length, and upwind fixation) did differ significantly between the respective test and control groups (Table 4.1). Only in the 'pure air stream' control, upwind length ($U = 112,\ P = 0.0214,\ n = 16$) and upwind fixation ($U = 107,\ P = 0.044,\ n = 16$) were significantly different from zero, i.e. the beetles showed a significantly negative anemotaxis when a pure air stream was presented. Without any air stream, the beetles showed no preference for any direction (upwind length: $U = 62,\ P = 0.782,\ n = 16$, upwind fixation: $U = 60,\ P = 0.706,\ n = 16$).

In the six-chamber-olfactometer, the adult beetles of *C. canaliculata* showed no clear preference for host plant odor. When the walking times above all six chambers were compared by the Friedman test, there were no significant differences (Figure 4.2). However, in the 'low quantity' setting, the beetles spent significantly more time above the chamber containing leaf material of their host *S. pratensis* than above the opposite control chamber ($W = 52,\ P = 0.010,\ n = 10$, Figure 4.2A). Both, when a 'high quantity' of leaf material was offered (Figure 4.2B) and in the 'upside-down' oriented six-chamber-olfactometer, where a medium leaf quantity was offered in the test chamber, the beetles were not attracted to the host plant odors (Figure 4.2C). In addition, the supposedly more natural 'upside-down' setting did not result in a discernible difference in beetle behavior.

In the stem arena without contact, beetles spent similar amounts of time on covered host plant stems [median (lower and upper quartile): 88.97 (55.19-184.4) s] and corresponding empty controls [94.27 (32.48-150.1) s] ($U = 99.5,\ P = 0.556,\ n = 18$).

4.3.2 Response to contact cues

When beetles could choose between stems of the host meadow sage (*S. pratensis*) and of the non-host yarrow (*A. millefolium*), adults of *C. canaliculata* spent significantly more time on the stems of their host plant (Figure 4.3A). However, the beetles did not discriminate between yarrow stems and dummies (toothpicks) (Figure 4.3B).

When the beetles could choose between dummies (cigarette filters) treated with one of the three host plant extracts of different polarity and dummies with the respective solvent alone, the beetles showed a significant preference for the *n*-hexane extract over controls (Figure 4.4A). The beetles did not show a significant preference for the other two extracts (dichloromethane and methanol; Figure 4.4B and C); however, a tendency towards a preference for the dichloromethane extract could be observed.

Table 4.1: Walking parameters of adult *Cassida canaliculata* beetles on the locomotion compensator. The median as well as the lower (25 %) and upper (75 %) quartile (in brackets) of the average walking speed, the straightness of walking (i.e. the quotient of vector length and total track length; ranging from 0 to 1), the upwind length (i.e. the vector length towards the odor source), and upwind fixation (i.e. the quotient of upwind length and total track length; ranging from -1 to +1) are given. P denotes the significance of the Mann–Whitney U-test. $n = 16$ beetles in all treatment groups.

Walking parameter	**Without air stream**	**Pure air stream**	**Air stream + *S. pratensis* leaves**	***P***	**Air stream + pot with only soil**	**Air stream + potted *S. pratensis* plant**	***P***
Walking speed (mm/s)	10.38 (8.91 to 11.48)	9.40 (8.38 to 10.24)	8.01 (6.24 to 9.66)	0.184	4.57 (3.17 to 6.75)	5.96 (3.85 to 8.24)	0.300
Straightness	0.94 (0.83 to 0.98)	0.85 (0.74 to 0.92)	0.83 (0.70 to 0.88)	0.468	0.71 (0.64 to 0.86)	0.76 (0.55 to 0.91)	0.780
Upwind length (mm)	-0.35 (-187.8 to 350.4)	-573.0 (-881.5 to -122.1)	16.25 (-538.6 to 342.0)	0.067	-125.7 (-424.3 to 5.13)	-234.7 (-509.4 to 162.1)	0.897
Upwind fixation	0.00 (-0.08 to 0.22)	-0.30 (-0.46 to -0.08)	0.01 (-0.27 to 0.21)	0.171	-0.19 (-0.38 to -0.01)	-0.16 (-0.29 to 0.17)	0.696

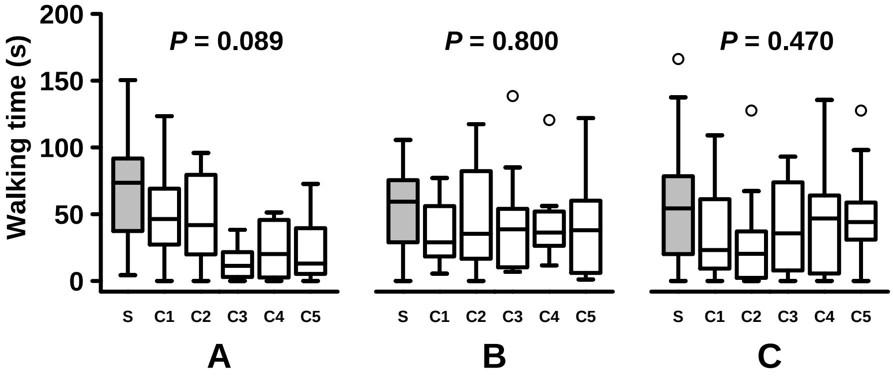

Figure 4.2: Response of adult beetles of *Cassida canaliculata* to volatile cues in the six-chamber-olfactometer: box-and-whisker plots of the walking time (in seconds) above the chambers containing leaf material of *Salvia pratensis* (S, gray box) as well as above the five empty control chambers (C1-C5, white boxes). Three different amounts of leaf material were tested: (A) one leaf (0.2-0.3 g; low quantity), (B) six leaves (2.5-3.4 g; high quantity), and (C) three leaves (0.8-1.8 g; medium quantity, the latter offered in an inverted set-up of the olfactometer). The boxes represent the median, and 25 % and 75 % percentiles. The whiskers extend to the maximum values; the circles denote outliers. $n_A = 10$ beetles, $n_B = 10$ beetles, $n_C = 15$ beetles. *P*-values of the Friedman test are given. The observation time was 5 min per beetle.

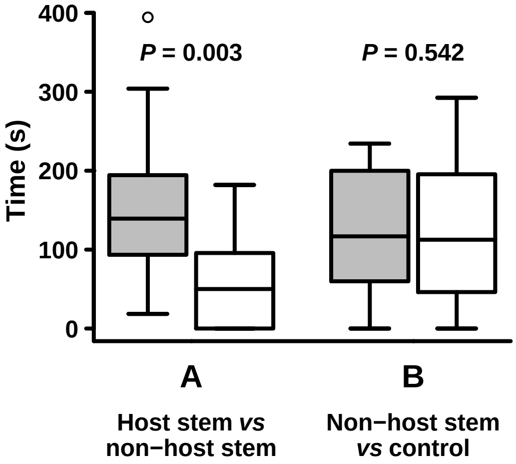

Figure 4.3: Response of adult beetles of *Cassida canaliculata* to contact cues (intact stems) in the stem arena: box-and-whisker plots of the time (in seconds) on (A) host stems (*Salvia pratensis*) *vs.* non-host stems (*Achillea millefolium*) and (B) non-host stems (*Achillea millefolium*) *vs.* control stems (toothpicks). The boxes represent the median, and 25 % and 75 % percentiles. The whiskers extend to the maximum values; the circle denotes an outlier. $n_A = 20$ beetles, $n_B = 18$ beetles. *P*-values of the Mann–Whitney *U*-test, comparing the mean relative difference in time spent on test and control stems to the null hypothesis of this difference being zero, are given. The observation time was 8 min per beetle.

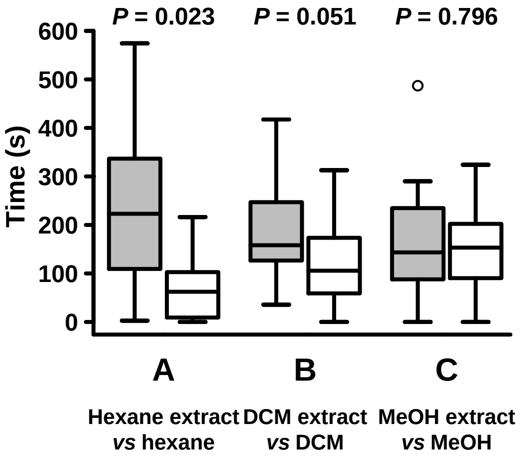

Figure 4.4: Response of adult beetles of *Cassida canaliculata* to contact cues (host plant extracts) in the stem arena: box-and-whisker plots of the time (in seconds) on test and control stem dummies (cigarette filters). (A) *n*-hexane extract *vs.* *n*-hexane, (B) dichloromethane (DCM) extract *vs.* DCM, and (C) methanol (MeOH) extract *vs.* MeOH. The boxes represent the median, and 25 % and 75 % percentiles. The whiskers extend to the maximum values; the circle denotes an outlier. $n_A = 18$ beetles, $n_B = 19$ beetles, $n_C = 17$ beetles. P-values of the Mann–Whitney U-test, comparing the mean relative difference in time spent on test and control stems to the null hypothesis of this difference being zero, are given. The observation time was 10 min per beetle.

The five fractions of the *n*-hexane extract did not reveal a significant response of the beetles, however, a slight tendency of a preference for fractions 1 (100 % *n*-hexane) and 3 (50 % *n*-hexane, 50 % dichloromethane) could be observed (Figure 4.5A-E). When the beetles were offered a 1:1 mixture of these two fractions compared with the respective mixture of solvents, the beetles significantly preferred this mixture to the control (Figure 4.5F).

4.4 DISCUSSION

Our results show that adults of the monophagous tortoise beetle *C. canaliculata* select their host plant chiefly by qualitative rather than quantitative contact cues. Although several other chrysomelid species are attracted to host plant volatiles (Feeny *et al.*, 1970; Andersen & Metcalf, 1986; Visser, 1986; Mitchell, 1994; Müller & Hilker, 2000, 2001; Kalberer *et al.*, 2001, 2005), olfactory cues seem to be only weak stimuli in the host plant recognition process of *C. canaliculata* that mainly moves by walking rather than flying.

On the locomotion compensator, where beetles could walk freely without any bar-

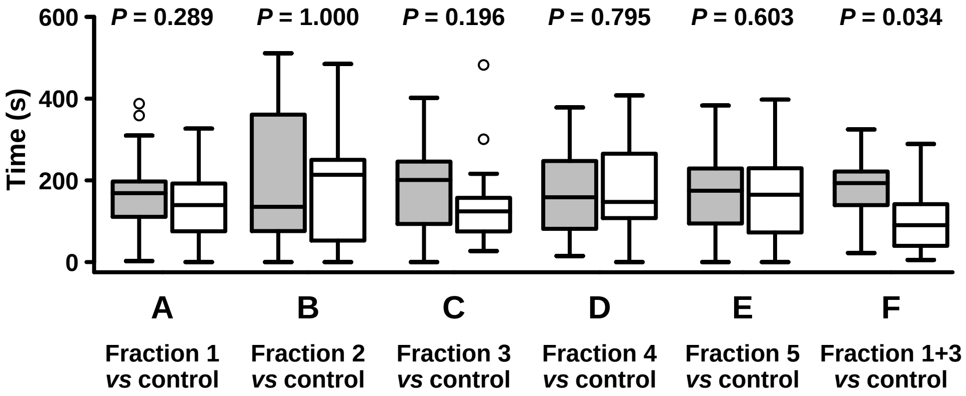

Figure 4.5: Response of adult beetles of *Cassida canaliculata* to contact cues (fractions of the *n*-hexane extract of *Salvia pratensis* leaves) in the stem arena: box-and-whisker plots of the time (in seconds) on test and control stem dummies (cigarette filters). The fractions were eluted in (1) 100 % *n*-hexane, (2) 90 % *n*-hexane + 10 % dichloromethane, (3) 50 % *n*-hexane + 50 % dichloromethane, (4) 90 % dichloromethane + 10 % methanol, and (5) 100 % methanol. The boxes represent the median, and 25 % and 75 % percentiles. The whiskers extend to the maximum values; the circles denote outliers. $n_A = 16$ beetles, $n_B = 13$ beetles, $n_C = 18$ beetles, $n_D = 18$ beetles, $n_E = 17$ beetles, $n_F = 18$ beetles. *P*-values of the Mann–Whitney *U*-test, comparing the mean relative difference in time spent on test and control stems to the null hypothesis of this difference being zero, are given. The observation time was 10 (A-E) or 8 min (F) per beetle.

rier in an air stream, they were not showing significant differences in neither of the analyzed walking parameters between the tested odor samples and the respective controls (Table 4.1). However, the beetles showed a negative anemotaxis when only a pure air stream was offered. While van Tilborg *et al.* (2004) argued that there is a selective advantage for searching upwind in the absence of odor, Sabelis & Schippers (1984) showed theoretically that upwind or downwind searching are equivalent strategies to find an odor plume. Thus, *C. canaliculata* may pursue the downwind searching strategy. When odors of *S. pratensis* leaves or whole plants were borne in an air stream, the beetles showed neither a positive nor a negative anemotaxis, however, a tendency of a difference ($P = 0.061$) could be seen comparing upward length between the ‘pure air stream’ control and the odor of *S. pratensis* leaves (Table 4.1). Possibly, the constant laminar airflow over the sphere may have resulted in a situation in which the beetle was ‘captured’ in the center of an infinite odor field. Therefore, it may have lacked information from any gradients in odor concentration and direction which could otherwise have elicited positive anemotaxis (van Tilborg *et al.*, 2003). Moreover, beetles might be sensitive to the strength of the air stream in which odors are borne. However, variation in the flow-rate (1000-3000 ml/min, data not shown) did not result in a changed walking behavior.

When plant volatiles were provided statically without air stream, beetles showed a slight attraction to odors from low amounts of leaf material (Figure 4.2A). While the median walking time above (Figure 4.2B) or below (Figure 4.2C) the chamber with high or intermediate amounts of leaves was also higher than on controls, these differences were not significant. The walking chamber of the olfactometer might have been already satiated with odor within the five minutes testing time when offering higher quantities of plant material. However, in the open stem arena, where the air could not satiate and beetles had very close access to plant parts, beetles were not attracted to their hosts. Thus, in total, we consider the response to volatile cues as rather weak.

In contrast, the beetles were very well able to discriminate their host plant based on contact cues when direct access to the stems was allowed in the stem arena. They clearly preferred stems of their host *S. pratensis* to stems of the non-host *A. millefolium* (Figure 4.3A). Moreover, the indiscriminative response to stems of *A. millefolium* compared to stem dummies (toothpicks) showed that the beetles were neither arrested by any (un)specific plant compounds nor deterred by this non-host plant.

Non-polar chemical stimuli were sufficient to cause arrestment in *C. canaliculata* (Figure 4.4A). Physical cues, i.e. the intact morphology of the stem surface, were not necessary for host plant recognition, since the beetles could discriminate dummies treated with host plant extract from dummies treated only with the solvent. This has been also found for another *Cassida* species (Müller & Hilker, 2001) and might be a general pattern within this genus. Nevertheless, physical cues might act additively or synergistically with contact cues in host recognition (Müller & Renwick, 2001; Müller & Riederer, 2005).

The ratio of compounds in the tested host plant extracts was probably quite different from the ratio that the beetles would perceive in nature. However, for the specialist *C. canaliculata* the presence of particular host plant compounds seems to be more important than a specific ratio of these compounds when direct contact is given. The use of qualitative cues such as the presence of single compounds or a mixture of specific compounds for host plant identification has also been reported from other specialized leaf beetles (Rees, 1969; Larsen *et al.*, 1992; Müller & Renwick, 2001). Thus, one might hypothesize, that in contact chemoreception the quantity, i.e. the relative ratio of compounds, is not as essential as in olfactory host plant identification, where the majority of herbivorous insects seem to respond only to very specific ratios of host plant volatiles (reviewed by Bruce *et al.*, 2005).

Moreover, we also found that one contact stimulus alone is not sufficient for host recognition in *C. canaliculata*. While individually tested fractions of the attractive *n*-hexane-extract did not reveal a significant response, a mixture of fraction 1 (100 %

n-hexane) and fraction 3 (50 % *n*-hexane + 50 % dichloromethane) was significantly arresting (Figure 4.5F). The fact that these two synergistically active fractions of the *n*-hexane extract were separated by an unattractive fraction (fraction 2) implies that there have to be at least two or more substances involved. The observed tendency of a preference towards the dichloromethane extract further supports this hypothesis, since this extract may have contained one of the active compounds also soluble in fraction 3. Thus, we can conclude that *C. canaliculata* needs at least two contact stimuli acting in concert to identify its host plant *S. pratensis*. Use of just one compound might be misleading if it is not entirely specific for *S. pratensis*. Comparing again contact to olfaction, ours and other results indicate that in both contact (Städler & Buser, 1984; Hopkins *et al.*, 1997; Müller & Renwick, 2001; van Loon *et al.*, 2002; Endo *et al.*, 2004; Tamura *et al.*, 2004) and olfaction (Fraser *et al.*, 2003; Bruce *et al.*, 2005) a blend of compounds can be necessary for host plant identification.

In the *n*-hexane extract, characteristic monoterpenes (e.g. β-pinene, 1,8-cineole) and sesquiterpenes (germacrene D, β-caryophyllene) of *S. pratensis* (Hegnauer, 1964; Veličković *et al.*, 2002) as well as typical components of cuticular waxes (Müller & Riederer, 2005) could be detected (D. Gabler and C. Müller, data not shown) that could potentially act as arrestants.

We also conducted some preliminary experiments on the importance of visual cues in host recognition by *C. canaliculata* (D. Gabler, unpublished data). As a first result of these experiments, it can be concluded that adults of *C. canaliculata* show visual orientation behavior. In the future, more elaborate experiments will be needed in order to elucidate whether the beetles are also able to recognize their host plant visually.

In summary, the results of this study indicate the following scenario for the host recognition process of the monophagous herbivore *C. canaliculata*. The beetles move mainly by walking rather than flying through their complex natural environment. As olfactory cues play only a weak role, beetles might meet their host plant most likely by random search. After contact evaluation, the presence of at least two distinct compounds is giving reliable information for arrestment at that plant. However, this implication has to be further elucidated with field experiments.

Chapter 5

Host plant finding in the specialized leaf beetle *Cassida canaliculata* – an analysis of small-scale movement behavior

with Sandra Ulmann, Elisabeth Obermaier, Oliver Mitesser and Hans Joachim Poethke

SUBMITTED TO ECOLOGICAL ENTOMOLOGY

Abstract. Host plant finding in walking herbivorous beetles is still poorly understood. Analysis of small-scale movement patterns under semi-natural conditions can be a useful tool to detect behavioral responses towards host plant cues that remain undiscovered in the laboratory.

In this study, the small-scale movement behavior of the monophagous leaf beetle *Cassida canaliculata* Laich. (Coleoptera: Chrysomelidae) was studied in a semi-natural arena (r = 1 m). In three different settings either a host (*Salvia pratensis* L., Lamiales: Lamiaceae; meadow sage), a non-host (*Rumex* sp., Caryophyllales: Polygonaceae; dock), or no plant was presented in the center of the arena.

While the beetles showed no differences in the 'general' walking parameters track length, straightness, and mean walking speed, the net distance from start- to end-point was significantly shorter in the host plant setting than in the two control settings. Moreover, the 'directional' parameters mean distance to the center and mean angular deviation from walking straight to the center were significantly smaller when a host plant was offered. Likewise, the angular deviation from walking straight to the center tended to decline with decreasing distance from the center. Finally, significantly more beetles were found on the host than on the non-host at the end of all trials.

It is concluded from these results that *C. canaliculata* is able to recognize its host plant from a distance. In contrast to laboratory experiments of an earlier study, the beetles can use olfactory (or a combination of olfactory and visual cues) to find their host, when an intact plant is presented under more natural conditions.

5.1 INTRODUCTION

Insects are able to use various cues for host plant finding (Jones, 1991; Bernays & Chapman, 1994; Bruce *et al.*, 2005). While the capability of insects to detect e.g. olfactory host plant cues has been studied in many flying and walking species (e.g. Feeny *et al.*, 1970; Visser & Avé, 1978; Blight *et al.*, 1995; Bartlet *et al.*, 1997; Kalberer *et al.*, 2001; Barata *et al.*, 2000; van Tol & Visser, 2002; Zhang & Schlyter, 2004; Bruce *et al.*, 2005; Kalberer *et al.*, 2005; Heisswolf *et al.*, submitted), the analysis of behavioral responses to such cues – and in particular the analysis of movement behavior – has been largely restricted to flying insects (Willis *et al.*, 1994; Guerrieri, 1996; Baker & Vickers, 1997; Witzgall, 1997; Vickers, 2000).

Most 'classical' experimental studies that try to elucidate which specific cues (e.g. different components or concentrations of odors) can be detected by a species use olfactometers, locomotion compensators, or wind tunnels within a laboratory (Böhm, 1995; McIntyre & Vaughn, 1997; van Loon *et al.*, 2000; Barata & Araújo, 2001; Tinzaara *et al.*, 2003; Kalberer *et al.*, 2005; Heisswolf *et al.*, submitted). However, these experiments often confine the behavioral repertoire of the animal under study. For example, on top of a locomotion compensator a beetle can choose to walk towards or away from the stimulus and it can change its walking pattern and speed. However, it is not allowed to change its position in relation to the stimulus. Consequently, such an experiment will not reveal potential differences in the behavioral response of the animal, which may be detected by an analysis of small-scale movement behavior in a more natural setting where the animal can move completely free. Although Jermy *et al.* (1988) already used a semi-natural arena experiment to analyze the movement behavior of the Colorado potato beetle *Leptinotarsa decemlineata* in the presence of host plants almost 20 years ago, such studies are still only rarely conducted due to the spatial and personnel requirements.

Recent laboratory experiments suggested that the strictly monophagous leaf beetle *Cassida canaliculata* Laich. (Coleoptera: Chrysomelidae) needs contact cues to identify its host *Salvia pratensis* L. (Lamiales: Lamiaceae), while it showed only a very weak reaction to olfactory cues in a six-chamber olfactometer and on a locomotion compensator (Heisswolf *et al.*, submitted). Additionally, it can be concluded from preliminary studies (D. Gabler, unpublished data) that *C. canaliculata* is able to use visual cues for orientation, but whether such cues are also important for host plant finding remains to be analyzed.

In the present study, an experimental design was developed, which allowed digital and electronic analysis of the movement patterns of *C. canaliculata* within a semi-natural arena. The data from the arena experiment were used to analyze if the movement pattern of this specialized species differed depending on whether (1) a host (*S. pratensis*), (2) a non-host (*Rumex* sp., Caryophyllales: Polygonaceae), or (3) no plant at all was placed in the center of the arena. Moreover, it was compared whether the beetles were more often found on the host than on the non-host plant during and at the end of the trials, i.e. whether *C. canaliculata* showed a preference for its single host plant *S. pratensis*.

5.2 MATERIAL AND METHODS

5.2.1 Species under study

The specialized tortoise beetle *Cassida canaliculata* Laich. (Coleoptera: Chrysomelidae) is strictly monophagous on meadow sage (*Salvia pratensis* L., Lamiales: Lamiaceae) (Wencker & Silbermann, 1866; Bourgeois & Scherdlin, 1899; Reitter, 1912; Graser, 1984; Trautner *et al.*, 1989; A. Heisswolf & D. Gabler, unpublished data), on which all developmental stages can be found. Within Germany, *C. canaliculata* is endangered, in Bavaria it is even threatened by extinction (Kippenberg, 2003); however, on warm slopes in the study area (Hohe Wann nature reserve in Northern Bavaria) it is locally abundant (A. Heisswolf & S. Reichmann, personal observations). Until now, little is published on the ecology of *C. canaliculata* (Steinhausen, 1949; Trautner *et al.*, 1989; Heisswolf *et al.*, 2005, in press).

Adults of *C. canaliculata* were collected in the Hohe Wann nature reserve in Northern Bavaria, Germany (50°03´N, 10°35´E) in May and June 2005. They were kept in boxes (115 x 115 x 61 mm) with a gauze lid (500 μm mesh) at room temperature and 16L:8D. The bottom of each box was covered with filter paper and the beetles were fed on leaves of *S. pratensis*.

The host plant meadow sage, *S. pratensis*, is very common in southern Germany. It is a perennial herb with a ground rosette that grows on dry meadows and field edges. The rosettes start to grow in late March and flowering begins in May (Schmeil & Fitschen, 1996). The non-host plant dock, *Rumex* sp. (Caryophyllales: Polygonaceae), is a very common ruderal plant all over Germany. It is a wintergreen perennial herb, also with a ground rosette, that grows in all kinds of meadows. Flowering lasts from May to August (Düll & Kutzelnigg, 2005). This plant was chosen as its growth form resembles that of *S. pratensis* but – belonging to a completely different plant order – it presumably emits a different blend of volatiles.

5.2.2 Arena experiments

The experiments were performed in a circular arena (r = 1 m) inside a greenhouse in the garden of the Field Station Fabrikschleichach (University of Würzburg). The bottom of the arena was covered with clay (1.5 cm deep). The edge of the arena (height: 30 cm) consisted of dark gray plastic, which was treated with Fluon® to prevent the beetles from escaping. There was a hole (diameter: 11 cm) in the center of the arena into which a pot could be placed.

The movement parameters of adult *C. canaliculata* individuals were recorded in three different settings, in which a potted host plant (*S. pratensis*, '*Salvia*'), a potted non-host plant (*Rumex* sp., '*Rumex*'), and a pot filled only with soil ('No Plant') was placed in the middle of the arena. In each setting, 24 beetles were released individually into the arena on a circle with a distance of 50 cm to the center of the arena. The release points were chosen randomly in 30° steps along this circle and each beetle was tested only once. The beetles were released in a supine position and the observation was started when the beetle had turned around on its own. Then, the location of the beetle was marked for 10 minutes in 30-second-intervals with consecutively numbered paper discs. When a beetle stayed motionless in a place for more than 30 seconds, only the numbered disc of the first interval was used as a marker. When the beetle had reached the plant, the distance to the center was defined as zero.

After each trial a digital photo (Sony CyberShot DSC-F828; 5 Megapixel) was taken of the movement path of each beetle. Prior to this, four white paper squares (each 10 x 10 cm) were placed in the arena with a distance of 10 cm to each other to be able to correct for distance differences and distortions. The photos were transferred to a PC and converted from 'jpg' to 'pgm' format with the ImageMagick 6.2.3-Q8 software (www.imagemagick.org). During this conversion, also the size of the photographs was reduced to 1296 x 972 pixels. The photographs were imported by the statistic software R 2.1.1 (R Development Core Team, 2005) using the 'pixmap' package (Bivand *et al.*, 2004). Then, the 21 marker discs, the center of the arena, as well as the four edges of one of the squares were consecutively clicked with the mouse to determine the x/y-coordinates of all of the points for further analysis. In the cases when a beetle had stayed in one place for more than one time interval, the marker disc was clicked as often as the number of time intervals the beetle had spent in this location. The four lengths of the paper square were averaged and were used as conversion factor from pixels to meters.

For the comparison of walking parameters between the three settings, the following parameters were calculated for each beetle: (1) track length (m), i.e. the sum of all step lengths, (2) net distance (m), i.e. the vector length from start to end point, (3) straightness, i.e. net distance / track length, (4) mean walking speed (cm/s), (5) mean distance to the center of the arena (m), and (6) mean angular deviation from walking straight to the center of the arena (°). For the calculation of this deviation only the absolute values were used, i.e. 0° denotes walking straight to the center, 90° stands for walking cross to the center, and 180° means walking straight away from the center.

For the analysis of changes in the walking pattern with the distance to the center of the arena, it was further calculated for each step: (7) the standardized walking speed, i.e. (walking speed per step - mean walking speed) / mean walking speed, and (8) the angular deviation from walking straight to the center (°).

5.2.3 Statistical analyses

Prior to analysis, all data points were removed, in which a beetle was on the plant or had contact with the edge of the arena. Then, all parameters were tested for normal distribution using the Shapiro–Wilk test. The three settings were compared using the Kruskal–Wallis H-test. When this test was significant, Mann–Whitney U-tests were used for pairwise comparisons between the groups. Regarding the null hypothesis that the beetles move completely randomly through the arena, the mean distance of all points within the arena to the center is $2/3$ m and the mean angular deviation from walking straight to the center is 90°. Thus, the parameters ‘mean distance from the center’ and ‘mean angular deviation from walking straight to the center’ were further compared to these critical values with the Mann–Whitney U-test. The number of beetles arriving at the host or the non-host, as well as the location of the beetles at the end of the trial (plant, arena, edge) was compared with the Fisher’s exact test. Relationships between the distance of a beetle from the center and its standardized walking speed as well as its angular deviation from walking straight to the center (cosine-transformed) were analyzed using Spearman rank-correlation. All statistical procedures were calculated with the software package R 2.2.1 (R Development Core Team, 2005).

5.3 RESULTS

5.3.1 Location during and at the end of each trial

During the trials, significantly more beetles visited the host plant (9 of 24) than the non-host (3 of 24) ($P = 0.047$). Moreover, all nine beetles stayed on the host plant while only one stayed on the non-host ($P = 0.005$; Figure 5.1). Neither the number of beetles that ended up at the edge of the arena ($P = 0.073$; Figure 5.1), nor the ones that were found anywhere in the arena did differ between the three settings ($P = 0.872$; Figure 5.1). In the setting, where a pot which was filled only with soil was placed in the center of the arena, the beetles were as often found at the edge of the arena as

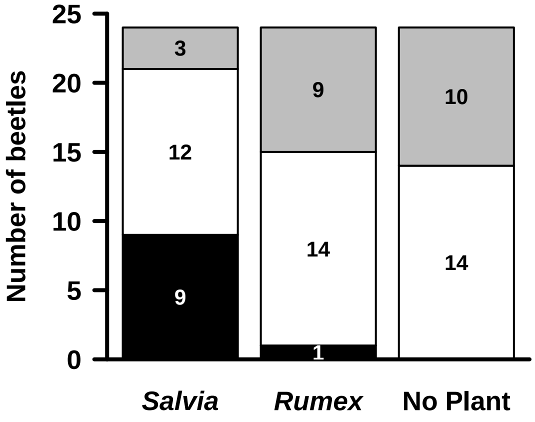

Figure 5.1: Distribution of *C. canaliculata* adults within a semi-natural arena at the end of the trials. A bar plot of the number of beetles which were found either on the offered plant (black), anywhere in the arena (white), or at the edge of the arena (gray) when either a host (*Salvia pratensis*), a non-host (*Rumex* sp.), or no plant at all was offered in the center of the arena. Each treatment group consists of $n = 24$ beetles. Results of the statistical analyses can be found in the text.

anywhere within the arena ($P = 0.387$; Figure 5.1).

5.3.2 Walking parameters

The ‘general’ movement parameters track length, straightness, and mean walking speed did not differ significantly between the three settings (Table 5.1). Only the net distance was significantly shorter when a host plant was offered in the center of the arena both compared to when a non-host plant ($U = 153$, $P = 0.005$, $n = 48$) or no plant at all ($U = 160$, $P = 0.008$, $n = 48$) was presented, while it did not differ significantly between the latter two settings ($U = 304$, $P = n.s.$, $n = 48$).

In contrast, the parameters which describe the direction of the beetles relative to the center of the arena, i.e. the mean distance to the center and the mean angular deviation from walking straight to the center of the arena, differed significantly between the three settings (mean distance: $H = 10.20$, $df = 2$, $P = 0.006$, $n = 24$ per setting; mean angular deviation: $H = 21.24$, $df = 2$, $P < 0.001$, $n = 24$ per setting). The mean distance to the center was significantly shorter when a host plant was offered compared to when either a non-host plant or no plant at all were offered (Figure 5.2). Likewise, the mean angular deviation from walking straight to the center was significantly smaller in the host plant setting than in the two control settings (Figure 5.3).

Only when a host plant was presented, the mean distance from the center was significantly smaller than the critical value of $2/3$ m ($U = 47$, $P = 0.001$, $n = 24$), while there was no difference when the non-host or no plant were offered (*Rumex*:

Table 5.1: 'General' walking parameters of *C. canaliculata* in a semi-natural arena when either a host plant (*Salvia pratensis*), a non-host plant (*Rumex* sp.), or no plant at all was offered in the center of the arena. Shown are the median and the 25 % and 75 % quantiles (in brackets) of the track length (m), i.e. the sum of all step lengths, the net distance (m), i.e. the vector length from the start to the end point, the straightness, i.e. the quotient of net distance and track length, and the mean walking speed (cm/s). P denotes the significance of the Kruskal–Wallis H-test. $n = 24$ beetles in each setting.

Walking parameter	***Salvia pratensis***	***Rumex* sp.**	**No plant**	***P***
Track length (m)	1.14 (0.62 to 2.12)	1.42 (0.83 to 2.04)	1.50 (1.15 to 1.89)	0.643
Net distance (m)	0.51 (0.49 to 0.69)	0.77 (0.55 to 1.09)	0.64 (0.55 to 1.14)	0.007
Straightness	0.41 (0.33 to 0.79)	0.56 (0.36 to 0.74)	0.49 (0.36 to 0.75)	0.949
Mean walking speed (m/s)	0.35 (0.26 to 0.48)	0.36 (0.21 to 0.41)	0.31 (0.24 to 0.36)	0.452

$U = 143$, $P = n.s.$, $n = 24$; no plant: $U = 165$, $P = n.s.$, $n = 24$). Moreover, the mean angular deviation from walking straight to the center was only significantly smaller than 90° when a host plant was offered ($U = 79$, $P = 0.021$, $n = 24$), while it was significantly greater than 90° when a non-host plant or no plant at all were presented in the center (*Rumex*: $U = 261$, $P < 0.001$, $n = 24$; no plant: $U = 294$, $P < 0.001$, $n = 24$).

Figure 5.2: 'Directional' walking patterns of *C. canaliculata* adults within a semi-natural arena. A box-and-whisker plot of the mean distance to the center (m) when either a host (*Salvia pratensis*), a non-host (*Rumex* sp.), or no plant at all was offered in the center of the arena. The boxes represent the median, and 25 % and 75 % percentiles. The whiskers extend to the maximum values; circles denote outliers. Different letters denote statistically significant differences ($P < 0.05$, Mann–Whitney U-test) between the groups. Each treatment group consists of $n = 24$ beetles.

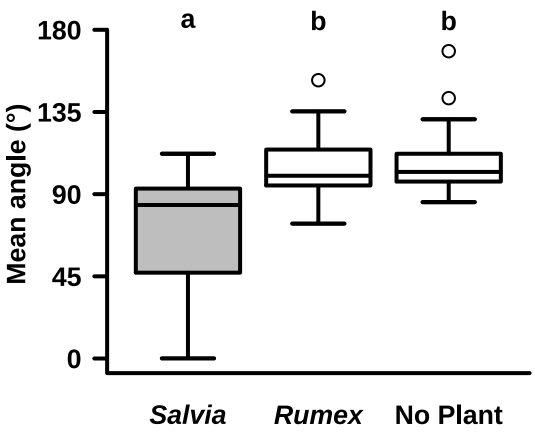

Figure 5.3: ‘Directional’ walking patterns of *C. canaliculata* adults within a semi-natural arena. A box-and-whisker plot of the mean angular deviation from walking straight to the center (°) when either a host (*Salvia pratensis*), a non-host (*Rumex* sp.), or no plant at all was offered in the center of the arena. The boxes represent the median, and 25 % and 75 % percentiles. The whiskers extend to the maximum values; circles denote outliers. Different letters denote statistically significant differences ($P < 0.05$, Mann–Whitney U-test) between the groups. Each treatment group consists of $n = 24$ beetles.

5.3.3 Walking parameters depending on distance from the center

The standardized walking speed was not significantly correlated with the distance from the center of the arena in none of the three settings (*Salvia*: $r_s = -0.110$, $P = 0.067$, $n = 280$; *Rumex*: $r_s = -0.079$, $P = 0.161$, $n = 318$; no plant: $r_s = -0.040$, $P = 0.456$, $n = 351$). When a host plant was offered, the angular deviation from walking towards the center of the arena showed a tendency of being correlated with the distance from the center of the arena ($r_s = -0.115$, $P = 0.054$, $n = 280$), i.e. the deviation tended to decline with decreasing distance to the center, however, the effect size was not very large. In the other two settings there was no such correlation (*Rumex*: $r_s = 0.006$, $P = 0.921$, $n = 318$; no plant: $r_s = 0.096$, $P = 0.073$, $n = 351$).

5.4 DISCUSSION

The majority of herbivorous insects have to find their host plants within a highly diverse mosaic of various non-host plant species. The host plant cues used in this process differ from species to species (Jones, 1991; Bernays & Chapman, 1994; Bruce *et al.*, 2005). A recent study suggested that olfactory cues play only a minor role in host plant finding by the monophagous leaf beetle *Cassida canaliculata* Laich., while the beetles are very well able to identify their host using contact cues (Heisswolf *et al.*, submitted). This implies that the beetles are not able to locate their host plant from a distance.

However, these experiments were performed under controlled laboratory conditions using a locomotion compensator, a six-chamber olfactometer, and a stem arena, which are possibly not sufficient to reveal potential differences in the beetles' behavioral response to plant stimuli. Thus, in the present study, a semi-natural arena experiment was developed, in which an intact host plant was offered to the beetles, which provided visual, olfactory, as well as contact cues simultaneously. There, a small-scale movement pattern analysis was used to re-examine the process of host plant finding in *C. canaliculata*.

Looking first at the distribution of beetles during and at the end of the trials, significantly more beetles were found on the host plant than when a non-host was presented and no beetles left the host plant once they had reached it, while two of three did leave the non-host. At first sight, this is only a confirmation of our previous laboratory results, which showed that *C. canaliculata*, is able to recognize its host *S. pratensis* using contact cues (Heisswolf *et al.*, submitted) and is no sufficient proof for host plant recognition from a distance. However, individual-based simulation studies (data not presented) showed that for all plausible movement parameters (the behavior of the beetles at the edge of the arena strongly influences the results) three (or more) hits in 24 random searching animals is well inside the range of a binomial distribution, while nine (or more) hits in 24 trials never reached a probability of more than 0.002 (with a repelling edge) and was an order of magnitude smaller in most other experimental setups. Thus, a detailed analysis of the 'general' and 'directed' walking parameters of *C. canaliculata* may shed more light on the host finding process in this species.

Regarding 'general' walking parameters, i.e. track length, straightness, and mean walking speed, no significant differences could be detected when the host (*S. pratensis*), the non-host (*Rumex* sp.), or no plant were placed in the center of the arena. Possible explanations could be that these 'general' parameters may be rather fixed in this species – as suggested by McIntyre & Vaughn (1997) for two *Eleodes* species – or that they are determined by the surface on which the beetles walked, which was the same in all settings. Likewise, the walking speed did not change systematically with the distance from the center. Regarding this observation, there is also no clear-cut null hypothesis to be found in the literature on the expected relationship between walking speed and the distance to a potential stimulus (visual or olfactory). For olfactory cues, both the hypothesis that insects increase their walking speed while approaching an attractive odor source as well as the hypothesis that they will walk more slowly in order to be able to locate the source are quite common (e.g. Thiéry & Visser, 1986; Bolter *et al.*, 1997; McMahon & Guerin, 2002). Thus, it remains unclear, whether the observed lack of correlation between the walking speed and the presence as well as the distance to the host plant is of any importance for the process of host plant finding.

The only ‘general’ parameter that was significantly different between the three settings was the net distance, which was significantly shorter in the host plant setting (0.51 m) compared to the other two settings (0.77 m and 0.64 m, respectively). However, this may be because all beetles were released in a distance of 0.5 m to the center of the arena, and thus, all beetles that reached the host plant (9 of 24) had a net distance of approximately 0.5 m. Thus, there may be no additional information gained from this result. Moreover, while there was no significant difference in the total track length, a shorter net distance should also lead to a higher straightness of walk. The fact that there was no statistically significant difference in this parameter may be a hint that the spatial scale, which was chosen for the analysis, was not fully appropriate. This could only be elucidated by using a finer spatio-temporal resolution of the beetles’ walking path.

Nevertheless, while the beetles showed no relevant differences in the ‘general’ walking parameters, the ‘directional’ parameters, i.e. mean distance to the center and mean angular deviation from walking straight to the center, differed significantly between the three settings: the beetles walked closer to the center and oriented more towards the center of the arena when a host plant was presented there. Likewise, the angular deviation from walking straight to the center tended to decrease with decreasing distance from the center. Although the effect size of these parameters was not very large, these results are clearly indicating that *C. canaliculata* is able to recognize its host plant from a distance. To control for a potential bias in the data that may stem from the fact that beetles who found the host plant did not leave it again and thus contributed to the data only with a movement towards the plant, further analyses were conducted in which these beetles were completely removed from the analysis. However, also in this case the differences in the ‘directional’ patterns were still significant between the ‘*Salvia*’ group and the two control groups (data not shown).

Our recent laboratory experiments (Heisswolf *et al.*, submitted) suggested that olfactory cues play only a minor role for *C. canaliculata*. However, the beetles may not have reacted to olfactory cues in the laboratory due to the artificial experimental conditions. Thus, it is possible that the beetles are well able to recognize olfactory host plant cues, when an intact plant is presented in a more natural setting. Additionally, they may use a combination of olfactory and visual cues, which has also been found in other herbivorous insects (McIntyre & Vaughn, 1997; Cook & Neal, 1999; Strom *et al.*, 1999; Barata & Araújo, 2001; Szentesi *et al.*, 2002). Regarding visual cues, it seems unlikely that *C. canaliculata* is able to recognize its host plant exclusively based on plant shape, as *S. pratensis* and *Rumex* sp. have a very similar growth form (A. Heisswolf, personal observation). Nevertheless, the spectral composition of plant tissue, which is used by some insects for host identification (Bullas-Appleton *et al.*, 2004; Fischer *et al.*, 2004), may differ between *S. pratensis* and *Rumex* sp. Additional experiments are needed to further elucidate the role of visual host plant cues in *C. canaliculata*.

In summary, although earlier laboratory experiments (Heisswolf *et al.*, submitted) yielded only weak evidence for a response of *C. canaliculata* to olfactory host plant cues, the results of the present study clearly show that the beetles are able to recognize their host *S. pratensis* from a distance. Within a semi-natural arena, the beetles walked closer to the center and oriented more towards the center of the arena when an intact host plant was presented there. Therefore, the analysis of movement patterns in a semi-natural environment is a useful tool to discover behavioral responses to host plant cues that could not be detected with standard laboratory methods.

Chapter 6

Selection of large host plants for oviposition by a monophagous leaf beetle: nutritional quality or enemy-free space?

with Elisabeth Obermaier and Hans Joachim Poethke

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Abstract. Oviposition site selection is crucial for the reproductive success of herbivorous insects. According to the preference–performance hypothesis, females should oviposit on host plants that enhance the performance of their offspring. More specifically, the plant vigor hypothesis predicts that females should prefer large and vigorously growing host plants for oviposition and that larvae should perform best on these plants.

The present study examined whether females of the monophagous leaf beetle *Cassida canaliculata* Laich. (Coleoptera: Chrysomelidae) prefer to oviposit on large host plant individuals of the meadow clary and whether large host plants are of higher nutritional quality than small host plants. Subsequently, it was tested whether the female preference correlates with offspring performance and survival.

In the field, females preferred large host plant individuals for oviposition and host plant quality, i.e. leaf nitrogen content, was significantly higher in leaves of large than of small host plants. In the laboratory, larval development time was shorter on leaves of large host plant individuals than on small host plant individuals, but this could not be shown in the field. However, a predator-exclusion experiment in the field resulted in a higher survival of larvae on large host plants than on small host plants when all predators had free access to the plants. On caged host plants there was no difference in survival of larvae between plant size categories.

It is concluded that females of *C. canaliculata* select oviposition sites that enhance both performance and survival of their offspring, which meets the predictions of the plant vigor hypothesis.

6.1 INTRODUCTION

In phytophagous insects, like leaf beetles, oviposition site selection is crucial for the successful development of larvae (Singer, 1986; Mayhew, 1997). In species that complete their whole development on a single host plant, host plant selection is even more essential (Singer, 1986; Craig *et al.*, 1989). Optimal oviposition theory (Jaenike, 1978) predicts that oviposition preference should correlate with host plant suitability for offspring development as females are assumed to maximize their fitness by ovipositing on high quality hosts (Awmack & Leather, 2002). This concept is often referred to as 'preference–performance hypothesis' (Jaenike, 1978). Oviposition site selection can be influenced by the quality or quantity of the host plant (Mattson, 1980; Strong *et al.*, 1984; Obermaier & Zwölfer, 1999). In this context, Price (1991) developed the 'plant vigor hypothesis', which is a special case of the preference–performance hypothesis. It predicts that vigorous plants that grow faster and ultimately reach a larger than average size should be preferred by the herbivore. Both plant biomass and nutritive quality, which are key factors for the development of herbivorous insects (McNeill & Southwood, 1978; Mattson, 1980; Strong *et al.*, 1984; Obermaier & Zwölfer, 1999), are supposed to be higher in vigorous plants than in other plants. The most important nutrient required by phytophagous insects is protein, which is usually the limiting factor for optimal growth (McNeill & Southwood, 1978; Mattson, 1980; Strong *et al.*, 1984; Crawley, 1989; White, 1993; Bernays & Chapman, 1994).

Positive correlations between oviposition site preference and offspring performance have been shown for different species (Craig *et al.*, 1989; Kouki, 1993; Preszler & Price, 1995; Howlett *et al.*, 2001; Craig & Ohgushi, 2002; De Bruyn *et al.*, 2002; Forister, 2004). However, many examples can also be found in the literature where females did not seem to prefer oviposition sites that would be best for the performance of their offspring (Rausher, 1979; Courtney & Kibota, 1990; Valladares & Lawton, 1991; Underwood, 1994; Berdegué *et al.*, 1998; Fritz *et al.*, 2000; Scheirs *et al.*, 2000, 2004; Faria & Fernandes, 2001; Harris *et al.*, 2001; Shiojiri & Takabayashi, 2003; van Nouhuys *et al.*, 2003). Thus, Scheirs & De Bruyn (2002) suggested integrating the two concepts of optimal foraging and optimal oviposition in plant–insect research. Optimal foraging theory predicts that phytophagous adults should prefer to feed on those hosts that give the highest adult performance (Stephens & Krebs, 1986). However, optimal adult and optimal offspring resources may be separated in space, and search-time constraints may therefore prevent the optimization of both strategies (Nylin & Janz, 1996; Krebs & Davies, 1997). Other approaches include the influence of higher trophic levels on oviposition site choice, as nutritionally inferior host plants may be chosen when they provide enemy-free space for the offspring (Hawkins *et al.*, 1993; Björkman *et al.*, 1997; Ballabeni *et al.*, 2001; Obermaier *et al.*, 2001; Singer & Stireman, 2003).

This study tested the preference-performance predictions of the plant vigor hypothesis (Price, 1991) in the monophagous leaf beetle *Cassida canaliculata* Laich. which feeds exclusively on meadow clary (*Salvia pratensis* L.). It was investigated whether offspring (i.e. larval) performance correlates with the oviposition site preference of the female beetles. Four questions were asked: (1) Do females prefer large-sized host plants for oviposition? (2) Do large host plants provide a higher nutritional quality, i.e. a higher leaf nitrogen content than small ones? (3) Do larvae perform better on large than on small host plants? (4) Do larvae have a higher chance of survival on larger than on small host plant individuals? The first question was addressed in a field study, questions 2 and 3 were investigated in a developmental study in the laboratory, while the last question was examined in a field experiment.

6.2 MATERIAL AND METHODS

6.2.1 Species under study

The monophagous tortoise beetle *Cassida canaliculata* Laich. belongs to the family of the leaf beetles (Coleoptera: Chrysomelidae, subfamily Cassidinae). With a body size of 9-11 mm *C. canaliculata* is the largest German tortoise beetle (Trautner *et al.*, 1989). Within Germany, the species is quite rare and found only on warm slopes in the southern part of the country. It is monophagous on *Salvia pratensis* L. (Lamiales: Lamiaceae), the meadow clary, on which all developmental stages can be found. The eggs are deposited in small clutches (8 ± 4 eggs per clutch) on the underside of the leaves and are covered with a secretion layer. Oviposition takes place from April to late June The host plant, *S. pratensis*, is very common in southern Germany. It is a perennial herb with a ground rosette that grows on dry meadows and field edges. The rosettes start to grow in late March and flowering begins in May (Schmeil & Fitschen, 1996).

6.2.2 Plant size categories

The host plants were divided into two size categories: large and small plants. Large plants were defined as plants that had an average ground rosette diameter of more than 20 cm and at least four vegetative cones. The category of small plants consisted of those plants that had an average ground rosette diameter of less than 10 cm and only one vegetative cone. This categorization was applied both to plants used in the laboratory as well as in the field study.

6.2.3 Oviposition site preference in the field

Adult oviposition site preference depending on the size of the host plant was studied at a mesoxerophytic grassland site (5000 m^2) in the Hohe Wann nature reserve in Northern Bavaria, Germany (50°03´N, 10°35´E). Within this site a 16 × 16 m^2 grid-plot of 64 subplots (2 × 2 m^2) was created. In 12 randomly selected subplots host plant size, i.e. average rosette diameter, and oviposition incidence were recorded for all 650 *S. pratensis* plants growing in these patches.

6.2.4 Larval development in the laboratory

For the laboratory experiments with larvae of *C. canaliculata* nine female/male pairs of field-collected adults were kept in separate boxes in a climatic chamber at a day temperature of 20 °C and night temperature of 16 °C and a LD 16:8 h photoperiod. Egg clutches were isolated and inspected daily for hatching larvae. The experiment started when at least 40 L1 larvae (not older than 24 h) were available. The larvae were randomly distributed into two treatment groups. In the first group (‘L group’) 20 larvae were reared on leaves of large plants of *S. pratensis*; in the second group (‘S group’) another 20 larvae were reared on leaves of small plants. The larvae were kept singly in plastic containers and were placed onto a single leaf. The leaves used for the feeding experiments were harvested at a mesoxerophytic grassland site in the Hohe Wann nature reserve where the leaf beetles occur naturally. Leaves were always collected at the same time of the day because of possible diurnal rhythms in the level of nitrogen. Inside the plastic boxes the leaves had a permanent water supply. After 4 days the leaves were replaced by new ones. For both treatment groups larval development time, pupal development time, and pupal weight were recorded. Leaf nitrogen content of the remaining leaf material was measured as a surrogate for host plant quality, as leaf nitrogen content is an adequate measure for protein content and as *S. pratensis* contains hardly any secondary plant compounds (Frohne & Jensen, 1985). The leaf material was dried at 70 °C for 48 h, ground in a mill (Retsch GmbH & Co. KG, Haan, Germany), and analyzed for per cent leaf nitrogen content in an elemental CHN analyzer (W. C. Heraeus Elemental Analysis, Hanau, Germany).

6.2.5 Larval survival in the field

The predator-exclusion experiment was conducted at a mesoxerophytic grassland site (6800 m^2) in the Hohe Wann nature reserve (see above for details). Sixty random points were generated on an aerial photo of the grassland site via the spatial analyst of the ArcView GIS software package (ESRI, Redlands, California). In the field, the random points were located with a portable GPS, and by the nearest-neighbor method 30 large and 30 small plants of *S. pratensis* were selected. This method may lead to a bias, as choosing plants by the nearest-neighbor method will tend to preferentially select plants growing in low densities over those growing in high densities. However, such a bias seemed negligible compared with the higher effort of a truly random sampling method. On each plant six L1 larvae were placed on the underside of one leaf. Large and small plants were each divided into three treatment groups. The first group (No Cage) received no treatment. All predators and parasitoids had free access to the larvae on the plants. In the two other groups, each plant was surrounded by a cage of $30 \times 30 \times 40$ cm^3 size. The cages consisted of a wooden frame that was covered with gauze (about 0.5 mm mesh-width). In the second treatment group (Open Cage) the cage was not completely closed: the top of the cage and a stripe of 5 cm width at the bottom of the cage were left open. This treatment group served as a control for existing cage effects due to changed microclimate within the cage. In the third treatment group (Closed Cage) the whole cage was covered with gauze to exclude all predators and parasitoids. The inner side of the bottom frame of the cages in the Closed Cage group was covered with a ring of glue to record larvae that tried to leave the plants. In all treatment groups the larvae were counted each second day until pupation. From these data, larval survival and larval development time could be determined.

6.2.6 Statistics

Adult oviposition site preference was tested with a logistic regression model. In order to evaluate the goodness-of-fit of the regression model the coefficient of determination R^2 after Nagelkerke (1991) was considered ($R^2_{Nagelkerke}$). *ROC* plots (receiver operating characteristics), i.e. the area under the resulting curve ('area under curve' = *AUC*), were used to determine the classification accuracy of the model (Hanley & McNeil, 1982, 1983; Zweig & Campbell, 1993). Developmental data were tested for normal distribution using the Kolmogorov–Smirnov test. t-tests and Mann–Whitney U-tests were used to compare the data on larval development between the two plant size groups. The proportion of larvae surviving to pupation were compared with binomial Generalized Linear Models (GLMs) and ANOVA. All statistical procedures were calculated with the software packages R 1.9.1 (R Development Core Team, 2004) and SPSS 12.0.2 (SPSS Inc., 2004).

6.3 RESULTS

6.3.1 Oviposition site preference in the field

The probability of oviposition increased highly significantly with the size of the host plant individuals (coefficient = 0.146, $R^2_{Nagelkerke} = 0.109$, $P < 0.001$, $AUC = 0.671$, $n = 650$, Figure 6.1).

6.3.2 Larval development in the laboratory

Larval development time was significantly shorter in the group of larvae reared on leaves of large plants (L group) compared with the larvae reared on leaves of small plants (S group) ($t = 6.559$, $P < 0.001$, $n = 38$). Pupal development time was, however, significantly longer in the L group than in the S group ($U = 84.50$, $P = 0.004$, $n = 38$). Total development time was again significantly shorter in the L group than in the S group ($t = 3.830$, $P < 0.001$, $n = 38$). There was no significant difference in pupal weight between the two treatment groups ($t = -1.747$, $P = n.s.$, $n = 38$). Per cent leaf nitrogen content was significantly higher in leaves of large plants than in leaves of small plants ($t = -9.455$, $P < 0.001$, $n = 38$). A summary of the results on larval development can be found in Table 6.1.

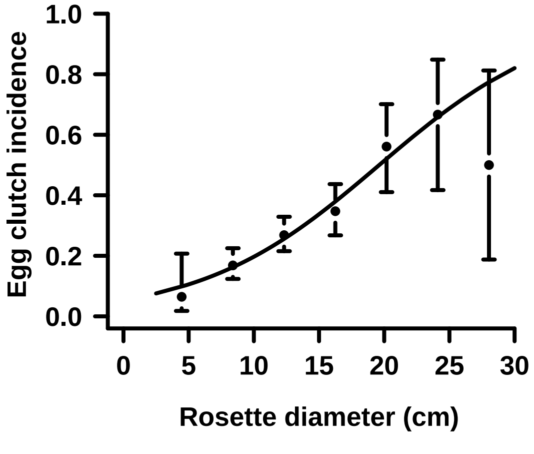

Figure 6.1: Oviposition site preference in *Cassida canaliculata* depending on host plant size. Plotted are the mean ± 95% CI of the egg clutch incidences in rosette diameter classes of 4 cm (circles with bars) and the predicted probability of egg clutch incidence (line) calculated by the logistic regression model, $n = 650$ plants.

Table 6.1: Larval development of *Cassida canaliculata* in the laboratory. Development time, pupal weight, and leaf nitrogen content are shown. One group of larvae was reared on leaves of large plants ($r > 20$ cm), the other group on leaves of small plants ($r < 10$ cm). $n = 19$ larvae in both treatment groups. Mean values ± SD and P-values of the t-test (t) or Mann–Whitney U-test (U) are given.

Parameter	Large plants	Small plants	*P*-value	
Larval development time (days)	25.0 ± 0.9	27.5 ± 1.4	< 0.001	(t)
Pupal development time (days)	11.2 ± 0.7	10.4 ± 0.7	0.004	(U)
Total development time (days)	36.2 ± 1.3	37.9 ± 1.4	< 0.001	(t)
Pupal weight (mg)	49.9 ± 5.4	47.2 ± 4.1	0.089	(t)
Leaf nitrogen content (%)	3.10 ± 0.22	2.48 ± 0.19	< 0.001	(t)

6.3.3 Larval survival in the field

In the No Cage treatment group a significantly higher proportion of larvae survived to pupation on large plants than on small plants ($z = 2.513$, $P = 0.012$, $n = 20$). In the group with ‘open cages’ there was no significant difference between the survival of larvae on large or small plants ($z = 1.237$, $P = n.s.$, $n = 20$). In the Closed Cage treatment group also, the survival of larvae did not differ between the plant size groups ($z = -0.204$, $P = n.s.$, $n = 20$). Comparing the survival of larvae within each plant size group, there was no significant difference between the No Cage and the Open Cage group on small plants ($z = 1.630$, $P = n.s.$, $n = 20$). However, there was a significant difference both between the No Cage and the Closed Cage group ($z = 3.151$, $P = 0.002$, $n = 20$) as well as between the Open Cage and the Closed Cage group ($z = -2.220$, $P = 0.026$, $n = 20$). On large plants there was no difference in larval survival between the three cage groups (ANOVA: $F_{df} = 0.5039$, $P = n.s.$, $n = 30$). A summary of the data can be found in Figure 6.2. Larval development time in the field did not differ significantly between the two host plant size groups ($t = -0.982$, $P = n.s.$, $n = 65$). The detailed larval development times of the three treatment groups are given in Table 6.2.

Table 6.2: Larval development of *Cassida canaliculata* in the field. Development time is shown for large and small host plants in the three treatment groups No Cage, Open Cage, and Closed Cage (A detailed description of the three cage groups is given in the text.) Mean values ± SD, number of larvae, and P-values of the Mann–Whitney U-test are given.

	Larval development time (days)				
Treatment	**Large plants**	*n*	**Small plants**	*n*	***P*-value**
No Cage	43.8 ± 4.3	12	45.0 ± 1.4	2	0.713
Open Cage	47.6 ± 3.7	11	46.6 ± 4.8	7	0.613
Closed Cage	44.9 ± 2.5	16	43.5 ± 2.9	17	0.141

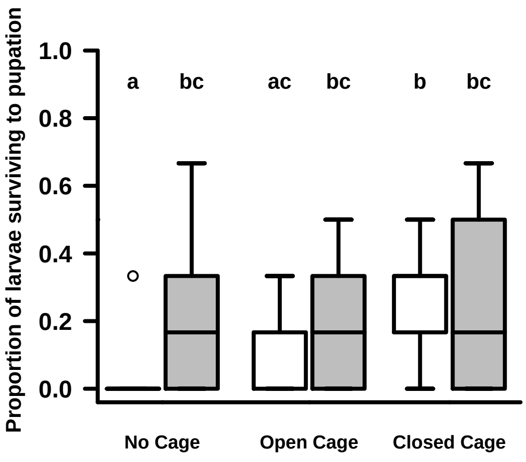

Figure 6.2: Larval survival of *Cassida canaliculata* on large and small host plant individuals in the field. A box-and-whisker plot of the proportion of larvae surviving to pupation in the three treatment groups No Cage, Open Cage, and Closed Cage is shown. (A detailed description of the three cage groups is given in the text.) White boxes represent small plants, grey boxes represent large plants. Each treatment group consists of $n_P = 10$ host plants with $n_L = 6$ larvae at the start of the experiment. The boxes represent the median, and 25% and 75% percentiles. The whiskers extend to the maximum values, circle denotes an outlier. Different letters denote statistically significant differences ($P < 0.05$) between the groups.

6.4 DISCUSSION

Although there are many reports of poor correlations between female oviposition site preference and offspring performance (reviewed by Mayhew, 1997), a strong oviposition-preference–offspring-performance relationship is still the basic assumption of most models describing oviposition site choice (Jaenike, 1978; Mayhew, 1997). Thus it is indispensable to collect more data on oviposition site choice and its consequences for adult and offspring performance. The plant vigor hypothesis (Price, 1991) – as a special case of the preference–performance hypothesis – predicts that females should prefer vigorously growing host plant individuals for oviposition, which should consequently enhance the performance of their larvae on those host plants.

The present study analyzed both the preference and the performance prediction of the plant vigor hypothesis and gives new support to this hypothesis and, more generally, to the preference–performance hypothesis (Jaenike, 1978). The results show that females of the monophagous leaf beetle *C. canaliculata* Laich. prefer to oviposit on large individuals of its host plant *S. pratensis* L. and the observed preference positively correlates with offspring (i.e. larval) performance: larvae of *C. canaliculata* had a significantly shorter development time on leaves of large *S. pratensis* plants in the laboratory. It is assumed that a faster development time is advantageous for the offspring. Possible advantages could be that a fast development might promote fitness in growing populations with overlapping generations, pupae might have priority in finding pupation sites, or males might achieve a higher mating success through a higher protandry. As these possible reasons are not the case in *C. canaliculata*, another advantage might be a higher chance of survival as predicted by the 'slow-growth–high-mortality hypothesis' (Feeny, 1976; Williams, 1999). This hypothesis suggests that, as slow-growing larvae spend a longer time in the more vulnerable, early stages of development than fast-growing larvae, they may suffer greater mortality from natural enemies and other mortality factors (Price *et al.*, 1980; Rhoades, 1983; Grossmueller & Lederhouse, 1985; Loader & Damman, 1991; Häggström & Larsson, 1995; Benrey & Denno, 1997). High developmental rates often correlate with high nutritional quality of the leaf material (Ohmart *et al.*, 1985; Lindroth & Hemming, 1990; Lindroth & Bloomer, 1991; Obermaier & Zwölfer, 1999). This was also the case in the present study. The percentage of total leaf nitrogen was significantly higher in leaves of large host plants compared with leaves of small host plants. There may be two extremes of coping with low quality food: (1) to have a constant development time and pupate at a lower than optimal weight, or (2) to opt for a prolonged development time to gain the optimal weight for pupation. In the case of *C. canaliculata* the second possibility seems to have been realized: there was no significant difference in pupal weight between the two treatment groups, but the larvae reared on leaves of small host plants (S group) – which were of poor nutritive quality – had a significantly longer development time than larvae reared on leaves of large host plants (L group). Contrary to this result, pupal development time was longer in the L group, yet total development time was still highly significantly shorter in the L group. Pupal development time may depend on the pupal weight or on the sex of the larvae. However, as already mentioned, there was no significant difference in pupal weight between the two treatment groups, neither was there any correlation between pupal weight and development time. As the sex ratio of the larvae was not recorded in the experiment, no conclusion can be drawn on this possible explanation.

According to the prediction of the slow-growth–high-mortality hypothesis (Feeny, 1976; Williams, 1999), a prolonged development time of larvae on leaves of small *S. pratensis* plants – as found in the laboratory – should lead to a higher mortality of larvae on small plants in the field. Indeed, the survival probability of larvae in the field was significantly higher on large than on small *S. pratensis* plants when all predators had free access to the plant. On plants where all predators were excluded there was no difference in survival between large and small host plants. However, development time in the field did not differ between larvae on small and on large plants. This may be due to the unstable weather conditions in the field compared with the constant setting in the climatic chamber where the developmental studies were conducted. The field conditions may consequently have led to a higher variability of larval time, which could have disguised any differences between larvae on large and small host plant individuals.

What could then explain the difference in mortality between the plant size groups? If survival is compared within the treatment groups, the only significant difference between large and small plants can be found in the No Cage group. Thus, at first sight one might think that the cages might have given protection from abiotic forces like wind and rain, resulting in a higher survival of the larvae. However, as the comparison within the plant size groups shows, there was no significant difference in survival between the No Cage and Open Cage group, which contradicts this first hypothesis. Thus, a more probable explanation is that large plants provide an enemy-free space (Jeffries & Lawton, 1984) for the larvae due to their size and their architectural complexity. As the larvae of *C. canaliculata* feed on the underside of the leaves of their host plant, they are not very conspicuous to predators. There are as yet no published studies on natural enemies of *C. canaliculata*, but it is reported that larvae of other tortoise beetles are predated by, for example, Coccinellid and Carabid beetles, Hemipteran bugs, ants, and spiders (Olmstead & Denno, 1993). On large host plants, which mostly have a higher number of leaves than small plants, predators may have to search for a longer time to find the larvae. In support of this hypothesis, it has been reported that a high structural complexity of plant architecture has a negative influence on the searching efficiency of parasitoids (Andow, 1990; Lukianchuk & Smith, 1997; Gingras *et al.*, 2002).

Another explanation for the loss of larvae could be that on small plants more larvae left the host plant to search for a higher quality plant. However, this hypothesis could not explain the observed pattern. If there were a difference in leaving tendency between small and large plants, this tendency should show in the Closed Cage group as well, because dispersing individuals would have been caught in the ring of glue before they reached the cage and might return to the plant. However, in the Closed Cage group (i.e. without any predator-caused mortality) there was no significant difference in larval survival between large and small plants, and only in two cases a single larva was found in the glue. Consequently there is no reason to assume a significant difference in leaving tendency between the plant size categories. On the other hand, there was a significant difference in larval survival between caged and uncaged experiments with small plants. It may therefore be concluded that the difference in larval survival on large and small host plants in the uncaged treatment group was due to predator-caused mortality. This would again favor the first explanation given here, assuming that the larvae can profit from an enemy-free space on large host plants.

Additionally, the results presented in this study give support to the ‘neural limitation hypothesis’ (Levins & MacArthur, 1969). This theory predicts that neural constraints result in a trade-off between diet breadth and the ability to discriminate among hosts, i.e. specialized species should be more capable of choosing between host plants of different quality than generalists (Janz & Nylin, 1997; Bernays, 1998, 2001). Thus, the ability of *C. canaliculata* – as a very specialized species – to choose host plants depending on quality meets the predictions of the neural limitation hypothesis.

Summing up the results, this study supports the performance prediction of the plant vigor hypothesis (Price, 1991) and therefore corroborates the preference–performance hypothesis (Jaenike, 1978). Larvae of the monophagous leaf beetle *C. canaliculata* performed better on large host plant individuals, which were also preferred as oviposition sites by female beetles. Larval development times were shorter on leaves of large plants in the laboratory. This could not be confirmed in the field; yet a higher proportion of larvae survived to pupation on large plants in the field. Further detailed information on predator- and non predator-caused mortality and on larvae that may possibly leave the plant are needed.

Chapter 7

From plant individuals to heterogeneous landscapes – environmental structure affects an herbivore-parasitoid interaction on multiple spatial scales

with Elisabeth Obermaier (First Author), *Hans Joachim Poethke, Barbara Randlkofer and Torsten Meiners*

SUBMITTED TO LANDSCAPE ECOLOGY

Abstract. Interactions between insect hosts and their parasitoids take place in a highly structured and complex environment. The heterogeneity of this environment is supposed to affect host-parasitoid interactions, but the quality and the strength of the impact may change with spatial scale. We investigated the influence of the environmental structure on the interaction between the tansy leaf beetle, *Galeruca tanaceti* L. (Coleoptera: Chrysomelidae), and its egg parasitoid, *Oomyzus galerucivorus* Hedqvist (Hymenoptera: Eulophidae), on four different spatial scales. 1) On the scale of the plant individual, plant architecture (height) had a positive influence on herbivore oviposition probability, and a negative impact on parasitism. 2) Likewise, on the microhabitat scale ($r = 0.1$ m around an oviposition site), the vegetation structure affected beetle egg deposition positively and egg parasitism negatively. 3) On the macrohabitat scale ($r \approx 20$ m), sites with a large proportion of shrub cover were avoided by herbivore and parasitoid. 4) On the landscape scale ($r = 100$-500 m around macrohabitats), herbivore and parasitoid were influenced positively by an increasing percentage of suitable habitat in the surrounding of habitat patches. While the structure of the environment affected both herbivore and parasitoid on all spatial scales examined, the host-parasitoid interaction itself was influenced only on the two smallest spatial scales, i.e. by the plant architecture and by vegetation structure in the direct vicinity of the oviposition site. This study therefore also contributes to recognize the importance of the integration of different spatial scales in ecological studies dealing with species interactions among different trophic levels.

7.1 INTRODUCTION

Host-parasitoid interactions do not take place in a homogeneous but in a highly structured and complex environment (Casas & Djemai, 2002). The structure of individual plants and the vegetation structure of habitat patches in general can influence the movement of herbivores (Crist *et al.*, 1992), their decision to enter a habitat (Cronin, 2003), their duration of stay (Bach, 1984), their life history traits (Langellotto & Denno, 2001), abundance (Anthes *et al.*, 2003; Dennis, 2004; Raghu *et al.*, 2004), and mortality (Kaitaniemi *et al.*, 2004). Structure can, however, also indirectly influence herbivorous insects via their natural enemies. On the scale of individual plants, Andow & Prokrym (1990) and Lukianchuk & Smith (1997) showed that a complex plant architecture did not only reduce the probability that egg parasitoids found host eggs, but it also functionally reduced the parasitoids' search time corresponding to the complexity of the plant. Gingras *et al.* (2002) drew the same conclusions from modelling the influence of plant structure on parasitism rate and from validating this in three natural tritrophic systems. Also on larger spatial scales vegetation structure influenced the probability that hosts and patches with hosts were found (Kemp & Simmons, 1978; Price *et al.*, 1980; Finke & Denno, 2002) as well as the abundance of their natural enemies (Langellotto & Denno, 2004). The examination of the importance of structural complexity for host-parasitoid interactions, however, has only recently set in (Casas & Djemai, 2002; Cronin, 2003; Meiners & Obermaier, 2004; Tschanz *et al.*, 2005).

The study of such interactions can be extended to even larger spatial scales up to the landscape level. The landscape structure is specified by different parameters than plant and vegetation structure. It represents, however, like habitat structure on smaller spatial scales, a measure for the heterogeneity of the environment and was shown to have a profound effect on the abundance and diversity of insect species in a given habitat (Thies & Tscharntke, 1999; Steffan-Dewenter & Tscharntke, 2002; Tscharntke *et al.*, 2002; Kruess, 2003). A growing body of literature supports the plea for landscape level analysis especially of host-parasitoid interactions (Hunter, 2002; Cronin & Reeve, 2005) as it emphasizes their role for the understanding of population and community ecology as well as for pest management.

Insect-plant and host-parasitoid interactions have long been one of the most actively studied fields in ecology. But the question of how the interplay between plants, their herbivores, and the natural enemies are affected by the spatial settings is a rather recent one and the inclusion of multi-species interactions in a spatial context has only just begun (Tscharntke *et al.*, 2002; Roslin & Kotze, 2005; Heisswolf *et al.*, in press). Even more, host-parasitoid interactions may be especially sensitive to scale effects, as for species belonging to different trophic levels different spatial scales may matter

(Wiens, 1989; Levin, 1992; Tscharntke & Brandl, 2004; Cronin & Reeve, 2005).

On any spatial scale, potential hosts of parasitoids can avoid parasitism by preferring oviposition sites where parasitoids are rare or absent (Quicke, 1997). The use of such an ‘enemy free space’ was defined by Jeffries & Lawton (1984) as ‘way of living that reduces or eliminates a species’ vulnerability to one or more species of natural enemies’ and was shown to exist for several systems (Berdegué *et al.*, 1996; Hopkins & Dixon, 1997; Gratton & Welter, 1999; Heisswolf *et al.*, 2005; Obermaier *et al.*, in press). Regarding plant and vegetation structure, herbivores may make use of enemy free space by oviposition on architecturally complex plants or within structurally complex vegetation where the risk of parasitism may be reduced.

The polyphagous tansy leaf beetle, *Galeruca tanaceti* L. (Coleoptera: Chrysomelidae), deposits its egg clutches in late autumn mainly on dry stalks of non-host plants within mesoxerophytic grassland sites. There, the eggs are exposed to the hymenopteran wasp *Oomyzus galerucivorus* Hedqvist (Hymenoptera: Eulophidae), the only egg parasitoid of *G. tanaceti* in the study area. We expected that a complex environmental structure serves as enemy-free space for the egg clutches of the herbivore. Plant structure was previously defined by its size, heterogeneity and structural complexity (plant form) (Andow, 1990; Gingras *et al.*, 2002). In this study, we extended this definition to larger spatial scales and categorized plant and vegetation structure due to their height (‘height of the structure’) or their ‘structural complexity’. On the landscape scale we defined landscape structure as ‘proportion of suitable habitat’ or as ‘biotope type diversity’ (Jonsen & Fahrig, 1997; Steffan-Dewenter & Tscharntke, 2002; Cronin & Reeve, 2005).

In a previous study, we investigated the influence of vegetation structure on the herbivore-parasitoid interaction on two spatial scales (micro- and macrohabitat, Meiners & Obermaier, 2004). In order to more completely understand the role of environmental heterogeneity for host-parasitoid interactions, we conducted an advanced study and analysis on the influence of plant architecture, and vegetation and landscape structure by extending our investigations to four spatial scales. On the level of the individual plant, microhabitat (r = 0.1 m and 0.5 m), macrohabitat, and the surrounding landscape (r = 100-500 m) we asked whether 1) the probability of herbivore oviposition increases and 2) the probability of parasitism decreases with increasing structural complexity on the respective spatial scale.

7.2 MATERIAL AND METHODS

7.2.1 Study system

The study was conducted on extensively managed meadows and mesoxerophytic grassland sites in diverse successional stages within the ‘Hohe Wann’ nature reserve (Northern Bavaria, Germany, 50°03´N, 10°35´E). The size of the nature reserve is approximately 1000 ha.

The tansy leaf beetle, *Galeruca tanaceti*, is polyphagous and feeds on species of the families Asteraceae, Brassicaceae, Caryophyllaceae, Dipsacaceae, Liliaceae, Lamiaceae, Polygonaceae, and Solanaceae (Lühmann, 1939; Prevett, 1953; Obermaier & Zwölfer, 1999). In the study area, one of the main host plants of *G. tanaceti* is yarrow, *Achillea millefolium* L. (Asterales: Asteraceae), (Meiners & Obermaier, 2004), but larvae can also be found feeding on *Centaurea jacea* L. (Asterales: Asteraceae) and *Salvia pratensis* L. (Lamiales: Lamiaceae) (E. Obermaier, personal observations).

In autumn, females of the tansy leaf beetle deposit their egg clutches on vertical structures within the herbaceous vegetation layer, mostly on non-host plants (E. Obermaier, personal observations), where the egg clutches then hibernate. The gravid females are unable to fly and have to walk up the plant structures for oviposition. After hatching in April-May, the larvae have to find suitable host plants in the surrounding of the oviposition site where they feed for about three weeks until pupation (Obermaier & Zwölfer, 1999). After pupation, the adults can be found from early June onwards before they enter a reproductive diapause in mid-summer.

The eulophid wasp *O. galerucivorus* parasitizes different *Galeruca* species (Sinacori & Mineo, 1993), however, its main host in Germany is the tansy leaf beetle. *O. galerucivorus* parasitizes the egg clutches of its host shortly after beetle oviposition in autumn. The parasitoid larvae hibernate in the host eggs and adults emerge next spring. The 1.5 mm long egg parasitoids search for host egg clutches by walking up and down vertical structures within the vegetation and using chemical cues from host feces (Meiners & Hilker, 1997; and unpublished data). To reach new sites with hosts they are (due to their small size) probably drifted by the wind.

7.2.2 Investigated scales

The scales on which organisms respond to environmental heterogeneity are determined by features of their behaviour, physiology, mobility, and life-history (Wiens, 2001). Different processes can be responsible for oviposition site selection in a plant-herbivore system at different spatial scales, like individual behaviour on the plant and the microhabitat scale and mode of dispersal, access and population persistence on the macrohabitat and the landscape scale (Wellnitz *et al.*, 2001). In our study system, on the plant and the microhabitat scale different habitats can be reached within a short time by walking females (*G. tanaceti* moves almost exclusively by walking within a site; E. Obermaier, personal observations). The egg parasitoid of *G. tanaceti* presumably also searches at close range for its host by walking (Hilker & Meiners, 1999). Colonization of the macrohabitats should be determined by the mobility and dispersal ability of the beetle individuals (flying and walking) and access to the sites. Additionally, shrub cover might influence access to oviposition sites for natural enemies in a negative (harder to find) or positive (more shelter and resources for parasitoids) way (Langellotto & Denno, 2004). Furthermore, on the landscape scale, occupation of macrohabitats by the beetle species was supposed to be also related to surrounding landscape parameters. We defined the spatial scales in this study according to the grain of the environmental factors, which we investigated. In order to use comparable parameters across different spatial scales we categorized ‘structure’ according to its height (‘height of the structure’) and its complexity (‘structural complexity’) and classed the parameters investigated on the different scales with these categories (Table 7.1). Only on the landscape scale we had to use different parameters (see below).

7.2.3 Parameters investigated on different spatial scales

7.2.3.1 Plant individual

Plant architecture and vegetation structure of the microhabitat were examined on two grassland sites called ‘Schafhof’ and ‘Holzplatz’. We created 80 (‘Schafhof’) and 40 (‘Holzplatz’) random points on aerial photos of the two sites using the Geographical Information Software Arc View GIS 3.2 (ESRI, Redlands, California). In the field, the random points were addressed with a portable GPS. At 60 (‘Schafhof’) and 20 (‘Holzplatz’) random points the point was shifted to the nearest plant with an egg clutch of *G. tanaceti* and this plant and egg clutch were selected for investigation. The remaining 20 random points per site were investigated as controls without egg clutches.

All selected plants with egg clutches were characterized for plant structural complexity. We registered for each plant whether it was a grass or herb and we counted the number of first order ramifications. To estimate the overall availability of plants of different architecture, we randomly selected 15 squares of 0.25 m^2 size per site and classified all plant individuals within these squares for whether they were grass or herb and counted their number of first order ramifications. Plant height, as an additional feature of plant architecture, had been examined in a separate investigation (Obermaier *et al.*, in press).

Table 7.1: Overview on parameters of the structure of the environment studied on different spatial scales for their effect on egg distribution of *G. tanaceti* and parasitism by *O. galerucivorus*. Structural parameters were categorized according to the height of the respective structure and the structural complexity of the plant or habitat patch (after Gingras *et al.*, 2002; modified for the different scales). Parameters, which are correlated significantly within a scale, are indicated by an asterisk (*) and are either summarized by principal components analysis (microhabitat) or partly excluded to avoid collinearity (macrohabitat). Scales and parameters indicated by numbers have been investigated in previous studies.

Structural parameters studied	
Height of structure	**Structural complexity**
Plant individual scale	
• Plant height [1]	• First order ramifications
	• Type of plant (grass/herb)
Microhabitat scale (r = 0.1 m)	
• Height of herbaceous layer *	• % Cover of herbs, grasses, and open ground
(average/maximal height)	• % Horizontal vegetation cover at 0.1 m intervals from 0 to 1.0 m *
	• Stalk number *
Macrohabitat scale [2] (r = 20 m)	
• Height of herbaceous layer [2]	• % Area covered by shrubs *, [2]
shrub layer *, [2]	
tree layer *, [2]	
Landscape scale (r = 100-500 m)	
• Biotope type diversity (Shannon Wiener diversity index *H*)	
• % Biotope types suitable for both the beetle and its parasitoid: 'suitable habitat'	
(fallow land, extensively managed meadows, mesoxerophytic grassland sites, and ruderal vegetation)	

[1] Obermaier *et al.* (in press)

[2] Meiners & Obermaier (2004)

7.2.3.2 Microhabitat

Around the egg clutch points as well as around the control points different measures of vegetation structure were recorded within a circle of 0.1 m radius. We registered the average height (i.e. the weighted mean vegetation height, after Sundermeier, 1999) and the maximal height of the herbaceous layer. We counted the number of plant stalks, and estimated the vertical cover of herbs, grasses and open ground. The horizontal vegetation cover was estimated in 0.1 m steps from 0 m up to 1.0 m using a white wooden board as background (Sundermeier, 1999). Additionally, plots were controlled for occurrence of yarrow, the main host plant of *G. tanaceti*.

As the parasitoids may not only be influenced by vegetation structure but also by the density of host egg clutches, we further determined egg clutch abundance of *G. tanaceti* in three radii of 1 m, 1.5 m and 2 m around the random points with egg clutch occurrence.

Finally, the focal egg clutches from the studied egg clutch points were collected from the two sites ('Schafhof': $n = 60$ and 'Holzplatz': $n = 20$) and were kept outdoors in the shade until the end of November. Then they were put singly in vials that were sealed with a fine net and kept for several weeks at room temperature until beetle larvae and adult parasitoids had hatched. The net was sprayed with water every three days to prevent egg clutches from drying out.

Parasitism was registered as incidence, i.e. whether at least one parasitoid had hatched from the egg clutch. To estimate the rate of parasitism per clutch, egg clutches of the site ‘Schafhof’ ($n = 60$) were dissected after parasitoids had hatched. Rate of parasitism was calculated as number of parasitoids hatched or completely developed within the eggs divided by the total number of eggs per egg clutch.

7.2.3.3 Landscape

For the study of the landscape structure the composition of biotope types surrounding 15 mesoxerophytic grassland sites with *G. tanaceti* occurrence was evaluated. Using the GIS, five circles with the radii 100-500 m were created around the centre of each site. From a biotope type map of the study area (J. Eibich, unpublished data) the percent area covered by the different biotope types (10 classes) was calculated for each circle. Landscape structure was analysed using two methods: (1) Landscape heterogeneity, i.e. habitat diversity, was calculated using the Shannon Wiener diversity index (H), and (2) the percentage of habitat types suitable for both the beetle and its parasitoid was determined. ‘Suitable habitat’ included the following biotope types: fallow land, extensively managed meadows, mesoxerophytic grassland sites, and ruderal vegetation. These biotope types were considered suitable, as they usually contain the main host plants of *G. tanaceti* and provide a sufficient nectar supply for the parasitoid. The remaining biotope types (fields, nutrient rich meadows, hedges, forest edges, forests, sealed areas) were assumed to be unsuitable. Herbivore egg clutch density was recorded along two transect lines which ranged each from one corner of the macrohabitat site to the other and crossed each other in the middle of the site. Thirteen egg clutches per site were collected and rate of parasitism (number of egg clutches parasitized per site / total number of egg clutches) determined. Herbivore egg clutch density and rate of parasitism on each macrohabitat site represented the dependent variables.

7.2.4 Statistics

On the spatial scales of individual plants, microhabitats, and surrounding landscape the influence of structural parameters was analyzed using multiple logistic regression (Jongman *et al.*, 1995; Hosmer & Lemeshow, 2000) or multiple linear regression (Sokal & Rohlf, 1995). Prior to analysis, collinearity of habitat variables was eliminated by principal components analysis. Models were accepted only if forward and backward logistic regression methods resulted in the same combination of habitat variables.

In order to evaluate the goodness-of-fit of the logistic regression models the coefficient of determination R^2 after Nagelkerke (1991) was considered. *ROC* (receiver operating characteristic) plots, i.e. the area under the resulting plot (area under curve = *AUC*), were used to determine the classification accuracy of the model (Zweig & Campbell, 1993; Guisan & Zimmermann, 2000; Bonn & Schröder, 2001). *AUC* values vary from 0.5 (determination by chance) to 1.0 (perfect classification). For model validation on the scale of the microhabitat, models were calculated for each site separately and then externally validated with the data of the other site (Fleishman *et al.*, 2003; Schröder & Reineking, 2004). The success of the validation was measured using the *AUC* value (Bonn & Schröder, 2001; Manel *et al.*, 2001). In other studies on habitat modelling, model accuracy and predictive success were shown to be substantially increased by using presence and absence values instead of only presence data (Brotons *et al.*, 2004) and detailed habitat data from the field instead of map-derived data (Berg *et al.*, 2004), both pre-requisites which are accomplished in the present study.

When multiple linear regression (stepwise) was used, all variables were tested for normal distribution prior to analysis and all variables consisting of percentage values were arcsin-transformed (Sokal & Rohlf, 1995). As above, collinearity of variables was avoided by principal components analysis. On the landscape scale data were evaluated using Spearman rank-correlations. All procedures were calculated with the software packages SPSS 11.0 and 12.0 (SPSS Inc., 2004).

7.3 RESULTS

7.3.1 Plant architecture: Herbivore oviposition

Egg clutches were found significantly more often on grasses than on herbs (‘Schafhof’: $\chi^2 = 16.2$, $P < 0.001$, $df = 1$, $n = 60$; ‘Holzplatz’: $\chi^2 = 15.0$, $P < 0.001$, $df = 1$, $n = 20$). However, if results were corrected for availability of these plant structures in the field, the effect of plant type on probability of oviposition of the beetle was no longer significant (‘Schafhof’: $\chi^2 = 0.40$, $P = n.s.$, $df = 1$). There was also no significant difference between the occurrence of egg clutches on structures with different numbers of ramifications and the availability of these structures (‘Schafhof’: $\chi^2 = 10.8$, $P = n.s.$, $df = 1$).

7.3.2 Plant architecture: Parasitism

No influence of plant architecture on probability of parasitism of an egg clutch could be found. Neither the number of first order ramifications per plant ($R^2_{Nagelkerke} = 0.030$, $P = n.s.$, $n = 60$) nor the type of the plant (herb or grass) ($R^2_{Nagelkerke} = 0.061$, $P = n.s.$, $n = 60$) had a significant influence on parasitism.

7.3.3 Vegetation structure in the microhabitat: Herbivore oviposition

On both sites the probability of oviposition of *G. tanaceti* significantly increased with increasing complexity of the vegetation structure around the oviposition site (‘Holzplatz’: coefficient $= 0.992$, $R^2_{Nagelkerke} = 0.225$, $P = 0.007$, $AUC = 0.775$, $P_{AUC} = 0.001$, $n = 40$, Figure 7.1a; ‘Schafhof’: coefficient $= 1.360$, $R^2_{Nagelkerke} = 0.215$, $P = 0.001$, $AUC = 0.768$, $P_{AUC} = 0.001$, $n = 80$, Figure 7.1b). The factor ‘vegetation structure’ consisted of the parameters maximal and mean vegetation height, number of stalks, and horizontal vegetation cover in 0.3 m height. i.e. beetle eggs were found more often in high and dense vegetation. As the model could be successfully cross-validated between both sites (Table 7.2), it seems to be of broader generality.

In contrast, the occurrence probability of *G. tanaceti* egg clutches was not related to the presence of the main host plant *A. millefolium* (yarrow) on the microhabitat scale. On the site ‘Schafhof’, yarrow was present in 91 % of the investigated microhabitats, on the site ‘Holzplatz’ in 50 %.

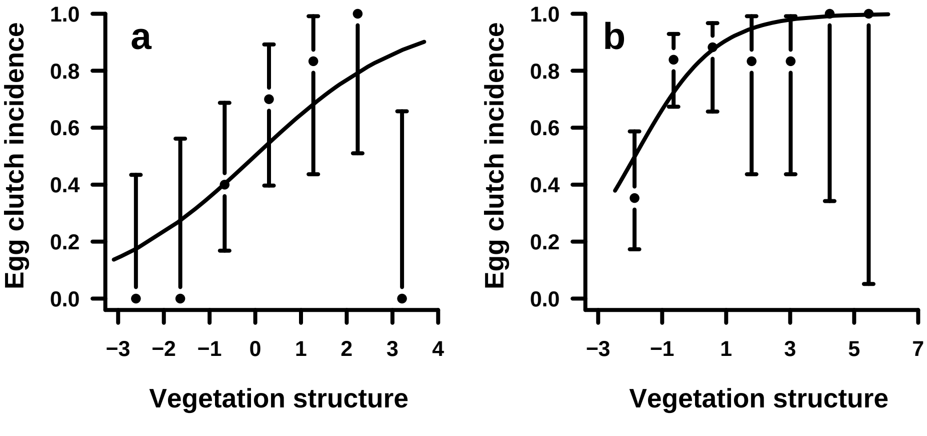

Figure 7.1: Oviposition of *G. tanaceti* in different vegetation structures (= factor composed of vegetation height (max, mean), % horizontal vegetation cover at 0.3 m height and stalk number) on (a) the site ‘Holzplatz’, $n = 40$ microhabitats and (b) the site ‘Schafhof’, $n = 80$ microhabitats. Plotted are the mean ± 95 % CI of egg clutch incidences (circles with bars) and the predicted probability of egg clutch incidence (line) calculated by the logistic regression model.

Table 7.2: Cross-validation of the logistic regression model explaining the egg distribution of *G. tanaceti* depending on microhabitat vegetation structure for the two sites 'Schafhof' and 'Holzplatz'. Models were parameterized with data from one site (training site, left column) and validated at the data from the other site (test site, first row). Given are *AUC* (± SE) and *P*-values of the validations. $n = 80$ microhabitats ('Schafhof') and $n = 60$ microhabitats ('Holzplatz').

Training/Test		**Schafhof**	**Holzplatz**
Schafhof	**AUC**	0.768	0.785
	SE	0.069	0.081
	P	0.001	0.010
Holzplatz	AUC	0.764	0.775
	SE	0.069	0.081
	P	0.001	0.010

7.3.4 Vegetation structure in the microhabitat: Parasitism

Parasitism could be investigated only on the site 'Schafhof' since none of the egg clutches collected on the second site was parasitized. There was a highly significantly negative correlation between the probability of parasitism and the factor 'vegetation structure' around the oviposition site (coefficient $= -1.154$, $R^2_{Nagelkerke} = 0.352$, $P = 0.001$, $AUC = 0.790$, $P_{AUC} = 0.001$, $n = 60$, Figure 7.2). In contrast, the probability of parasitism increased with increasing density of *G. tanaceti* egg clutches within a radius of 2 m around the oviposition site (coefficient $= 0.431$, $R^2_{Nagelkerke} = 0.140$, $P = 0.010$, $AUC = 0.700$, $P_{AUC} = 0.007$, $n = 60$). The factor 'vegetation structure' consisted of the same parameters as above. The rate of parasitism of *G. tanaceti* egg clutches was also significantly negatively correlated with vegetation structure (coefficient $= -0.162$, $R^2_{corrected} = 0.110$, $P = 0.006$, $n = 60$) and showed a tendency of a positive correlation with host density (coefficient $= 0.064$, $R^2_{corrected} = 0.05$, $P = 0.051$, $n = 60$).

The results suggest that the likelihood that parasitoids found the beetles' egg clutches decreased with increasing density and height of the vegetation around the oviposition site while it increased with increasing density of *G. tanaceti* egg clutches.

7.3.5 Landscape structure: Herbivore oviposition

The structure of the surrounding landscape (habitat diversity and percentage of suitable habitat) in the five radii (100-500 m) around the studied habitat patches had no influence on the overall occurrence of the leaf beetle in the habitat patches. Likewise, habitat diversity was not correlated with egg clutch density in none of the radii. There was, however, a trend of positive correlations of egg clutch density with the proportion of suitable habitat (fallow land, extensively managed meadows, mesoxerophytic grassland sites, and ruderal areas) within all five radii. The correlation was significant

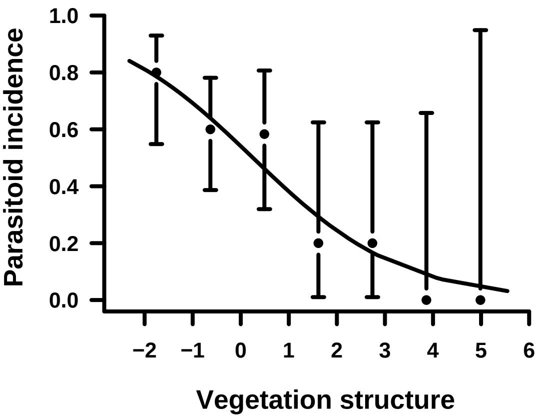

Figure 7.2: Parasitism of egg clutches of the leaf beetle *G. tanaceti* by the parasitoid *Oomyzus galerucivorus* at different vegetation structures. Plotted are the mean ± 95 % CI of parasitoid incidences (circles with bars) and the predicted probability of parasitoid incidence (line) calculated by the logistic regression model, n = 60 microhabitats.

in a radius of 200 m around the sites (Table 7.3).

7.3.6 Landscape structure: Parasitism

Habitat diversity was also not correlated with probability or rate of parasitism within the five radii around the studied habitat patches. However, probability and rate of parasitism on the focal sites again tended to increase with an increasing proportion of suitable habitat in the surrounding of the sites. The correlation between the proportion of suitable habitat and rate of parasitism was significant in a radius of 500 m (Table 7.3), while the correlation with the probability of occurrence of the parasitoid on

Table 7.3: Correlations between the proportion of suitable habitat area for *G. tanaceti* and *O. galerucivorus* (fallow land, extensively managed meadows, mesoxerophytic grassland sites, and ruderal vegetation) and density of beetle egg clutches or rate of parasitism at five different radii (100-500 m) around habitat patches at the landscape scale. Given are the correlation coefficients (r_s) and P-values of Spearman rank-correlations. $n = 15$ sites.

	Egg clutch density		**Parasitism rate**	
Radius	r_s	P	r_s	P
100 m	0.49	0.07	0.40	0.13
200 m	**0.57**	**0.03**	0.37	0.16
300 m	0.43	0.11	0.30	0.26
400 m	0.44	0.10	0.48	0.06
500 m	0.44	0.10	**0.57**	**0.02**

Table 7.4: Univariate logistic regression models explaining the occurrence of *O. galerucivorus* depending on the proportion of suitable habitat area (fallow land, extensively managed meadows, mesoxerophytic grassland sites, and ruderal vegetation) at five different radii (100-500 m) around habitat patches at the landscape scale. Given are the regression coefficient (b), P-value, $R^2_{Nagelkerke}$ (R^2_N), AUC-value, and P_{AUC} of the logistic regression model. $n = 15$ habitat patches.

Radius	b	P	R^2_N	AUC	P_{AUC}
100 m	0.061	n.s.	0.282	0.767	n.s.
200 m	0.089	0.050	0.291	0.833	0.030
300 m	0.093	n.s.	0.288	0.767	n.s.
400 m	0.164	0.010	0.462	0.867	0.017
500 m	0.167	0.009	0.479	0.867	0.017

the habitat patches was significant within three (200, 400, and 500 m) of five radii on the landscape scale (Table 7.4).

7.4 DISCUSSION

As insect herbivores depend primarily on their host plants for survival and reproduction, analyses of the abundances and distribution patterns of these plants have been the basis of many studies on herbivore-plant interactions (Bernays & Chapman, 1994). Recently it has been emphasized, however, that this approach may be too restricted and habitats for insect herbivores have been defined too narrowly. In particular, non-consumable resources (structural elements) have been ignored (Dennis, 2004). The present study reveals that plant and vegetation structure cannot only influence distributions of herbivores or parasitoids, but even seems to have the potential to mediate interactions across these different trophic levels (host-parasitoid interactions). This depends, however, on the spatial scale examined. Table 7.5 gives a schematic overview on the influences of plant, vegetation, and landscape structure on the host-parasitoid system for all spatial scales analyzed in the present and two earlier studies (Meiners & Obermaier, 2004; Obermaier *et al.*, in press).

On the scale of individual plants, plant architecture was defined as size, heterogeneity and structural complexity of a plant (Andow & Prokrym, 1990; Gingras *et al.*, 2002). In the literature especially structural complexity had been shown to affect herbivores (Raghu *et al.*, 2004) as well as parasitoids (Andow & Prokrym, 1990; Lukianchuk & Smith, 1997). For example, host finding success in parasitoids was higher on simply structured plants than on plants with complex structures (Andow, 1990; Lukianchuk & Smith, 1997; Gingras *et al.*, 2002, 2003). In this study, however, structural complexity of the plant individual (number of ramifications of a plant or plant type (grass/herb)) neither had a significant influence on the egg distribution of the tansy leaf beetle nor on the probability of parasitism by *O. galerucivorus* when

Table 7.5: Overview on the influence of plant architecture, and vegetation and landscape structure on herbivore oviposition and parasitism of egg clutches of *Galeruca tanaceti*. + denotes a significantly positive relationship, – stands for a significantly negative relationship, n.s. means not significant. On the scales and for the parameters where structural complexity has an opposite influence on the herbivore and its parasitoid it possibly mediates their interaction.

Scale	**r**	**Variable**	**Herbivore oviposition**	**Parasitism**
Plant individual	–	first order ramifications	n.s.	n.s.
	–	plant height [1]	+ [1]	– [1]
Microhabitat	0.1 m	'vegetation structure' [a]	+	–
	0.5 m	'vegetation structure' [b, 2]	+ [2]	n.s. [2]
Macrohabitat	20 m	% area covered by shrubs [2]	– [2]	– [2]
Landscape	100 m	% suitable habitat	n.s.	n.s.
	200 m	% suitable habitat	+	n.s.
	300 m	% suitable habitat	n.s.	n.s.
	400 m	% suitable habitat	n.s.	n.s.
	500 m	% suitable habitat	n.s.	+

[1] Obermaier *et al.* (in press)

[2] Meiners & Obermaier (2004)

[a] Factor 'vegetation structure' obtained by principal components analysis, consisting of height of herbaceous layer (max, mean), % horizontal vegetation cover at 0.3 m height, stalk number

[b] Factor 'vegetation structure' obtained by principal components analysis, consisting of height of herbaceous layer (max, mean), % horizontal vegetation cover at 0.1 m, 0.3 m, 0.5 m height

the abundance of the plant types in the field was taken into account. A possible explanation for this lack of evidence could be that plant individuals in this study system might not be selected due to their structural complexity, but because of other plant architectural traits like plant size. Supporting this hypothesis, we could show in a previous study (Obermaier *et al.*, in press) that oviposition height of the tansy leaf beetle was positively correlated with height of the plants the egg clutches were attached to, and that beetles selected the highest plants for oviposition. This may be explained by the fact that eggs that were deposited high above the ground suffered significantly less from parasitism and winter mortality than egg clutches at lower oviposition sites (Obermaier *et al.*, in press). Thus, choosing high oviposition sites within the herbaceous vegetation may result in a higher selective advantage for the females of this beetle species than ovipositing on plants with a high number of ramifications.

Also on larger spatial scales we hypothesized that complex vegetation structure might provide enemy free space for insect herbivores. In several studies vegetation height and structural complexity were shown to affect herbivore species richness (Stinson & Brown, 1983), population density (Dennis, 2004), habitat colonization (Coll & Bottrell, 1994), mate finding (Langellotto & Denno, 2001), and host plant detection (Anthes *et al.*, 2003). Regarding natural enemies the effect of vegetation structure varies. An investigation on larval predation and egg parasitism in the shield beetle species *C. rubiginosa* revealed a higher predation risk of larvae on exposed host plants, but no influence on egg parasitism or oviposition site choice of the female beetles (Tschanz *et al.*, 2005). On the other hand, in a meta-analysis, seven out of nine natural enemy guilds turned out to be most abundant under conditions of increased habitat complexity, with hunting spiders and web-building spiders showing the strongest response, followed by hemipterans, mites, and parasitoids (Langellotto & Denno, 2004).

In the present study, vegetation structure had a highly significant influence on both the egg distribution of the herbivore and parasitism by *O. galerucivorus* on the microhabitat scale, i.e. in the close surroundings of the oviposition site. While beetle egg clutches were found more often in plots with high and dense vegetation, their risk of being parasitized was higher in low and loose vegetation as both occurrence and rate of parasitism were negatively correlated with height and density of the surrounding vegetation. The model explaining egg clutch occurrence depending on the factor 'vegetation structure' could both be successfully cross-validated between two study sites and confirmed results of a previous study conducted on a slightly larger spatial scale (r = 0.5 m) (Meiners & Obermaier, 2004; Table 7.5). 'Vegetation structure', combining the parameters stalk number, horizontal cover, and average and maximal vegetation height per plot, seemed to provide enemy free space for the beetle females to hide their eggs from the parasitoids. This is in accordance with optimal oviposition theory, which predicts that females should oviposit at those sites that enhance the performance and survival chances of their offspring (Jaenike, 1978; Thompson, 1988; Thompson & Pellmyr, 1991; Heisswolf *et al.*, 2005). We suspect that in high and dense vegetation parasitoids show a shorter giving-up time and switch earlier to other patches, since the parasitoid searches the egg clutches of its host by running up and down vertical structures (T. Meiners, unpublished data).

Additionally, the probability of parasitism was positively correlated with host density (positive density dependence), i.e. depositing too many eggs in dense vegetation may spoil the selective advantage of hiding eggs in dense vegetation. A positive density dependence of parasitism on this scale corresponds quite well with earlier results on the sensory capabilities of a close congener of the egg parasitoid examined, *O. gallerucae*, which locates the microhabitat of its host by odors and contact cues (kairomones) of host feces (Meiners & Hilker, 1997). Plots with high egg clutch density may increase the residence time of parasitoids and thus their probability of finding host eggs, as attractive odors may be more intensive and contact with beetle feces may occur more frequently.

On the macrohabitat scale, the influence of vegetation structure, measured as percent shrub cover per site, was examined in an earlier study with the same study system

(Meiners & Obermaier, 2004). We hypothesized that habitat patches with an overall more dense vegetation could provide increased enemy free space for the beetle individuals. However, a high percentage of shrub cover significantly reduced both the probability of oviposition of the leaf beetle and the rate of parasitism within a site. Therefore, on this spatial scale, complex vegetation structure did not provide enemy free space for the beetle eggs, but was avoided by both the herbivore and the parasitoid. Concerning *G. tanaceti*, these results were interpreted as possible trade-off between vegetation structure, host plant availability, and microclimate. We suggested that host plant availability was higher and microclimate warmer at sites with less shrub cover. Sites in earlier successional stages might therefore be more suitable for the herbivore, in spite of a higher parasitism risk. Parasitism was, like on the microhabitat scale, positively density dependent.

Environmental heterogeneity can have a tremendous effect on host-parasitoid interactions on a large spatial scale (Cronin & Reeve, 2005). A study by Thies & Tscharntke (1999) for example showed that parasitism of *Meligethes aeneus* was higher in structural complex landscapes than in simply structured landscapes with a high proportion of agricultural land use.

In the studied system, habitat diversity of the surrounding landscape *per se* did neither have a significant influence on the host nor on the parasitoid. However, the percentage of suitable habitat in the surrounding landscape was positively correlated with herbivore egg clutch density, as well as with occurrence and rate of parasitism within the studied habitat patches. The number of parasitoids and therefore both probability and rate of parasitism might increase with an increasing proportion of habitat area in the landscape around sites because of the positive density dependence of the parasitoid (shown on smaller spatial scales). This hypothesis could be supported by results of Bergman *et al.* (2004) who emphasized the importance of the amount of suitable habitat area in the surrounding landscape for several butterfly species.

The rareness of studies on the influence of structural complexity on ecological interactions across multiple spatial scales in the landscape (Thies & Tscharntke, 1999; Steffan-Dewenter & Tscharntke, 2002; Kruess, 2003) stresses the need of further research in this area (Hunter, 2002). Kruess & Tscharntke (2000) could show that herbivorous insects and their natural enemies react on different spatial scales to the heterogeneous structure of the landscape, as the relevant spatial scale seems to be closely connected to the trophic level of the species under study (Tscharntke & Brandl, 2004). In our data set the range of spatial scales on the landscape scale might have been too small to reveal such differences across trophic levels.

The relevant spatial scales of the present study, on which environmental heterogeneity seemed to mediate the interaction between the leaf beetle *Galeruca tanaceti* and its egg parasitoid *Oomyzus galerucivorus*, were the two smallest scales, the plant individual and the close surrounding of the oviposition site (r = 0.1 m) (Table 7.5). At these scales the beetles were able to ‘hide’ their eggs from parasitism by ovipositing on tall plants high above the ground, and in plots of high and dense vegetation. On all other spatial scales the herbivore and the parasitoid were affected in the same way by vegetation or landscape structure.

Chapter 8

Enemies in low places – insects avoid winter mortality and egg parasitism by modulating oviposition height

with Elisabeth Obermaier (First Author), *Barbara Randlkofer and Torsten Meiners*

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Abstract. Oviposition site selection in insects is essential in terms of low egg mortality, high offspring survival and therefore a high reproductive output. Although oviposition height could be a crucial factor for the fitness of overwintering eggs, it has rarely been investigated.

In this study the oviposition height of a polyphagous leaf beetle, *Galeruca tanaceti* Linnaeus, in different habitats and at different times of the season was examined and its effect on egg clutch mortality was recorded. The leaf beetle occurs as an occasional pest on several agricultural plants. It deposits its eggs within herbaceous vegetation in autumn. Eggs are exposed to numerous biotic and abiotic mortality factors summarized as egg parasitism and winter mortality. Oviposition height of the leaf beetle was not uniform, but changed significantly with the structure of the habitat and during the season. Mean oviposition height per site (70.2 ± 4.9 cm) was significantly higher than mean vegetation height (28.4 ± 2.4 cm). Height of plants with egg clutches attached and oviposition height were significantly positively correlated.

The results suggest that females try to oviposit as high as possible in the vegetation and on the plants selected. In accordance with this, the probability of egg parasitism and of winter egg clutch mortality significantly declined with increasing oviposition height. A preference of *G. tanaceti* for oviposition sites high up in the vegetation might therefore have evolved due to selection pressures by parasitoids and winter mortality.

8.1 INTRODUCTION

Being immobile, insect eggs are the most vulnerable and endangered of all developmental stages (Hilker, 1994). The selection of the optimal oviposition site can be essential for female fitness (Scheirs & De Bruyn, 2002; Scheirs *et al.*, 2004) and should be optimized in terms of a high offspring survival and low egg mortality.

Egg mortality of herbivorous insect eggs can be caused, for example, by parasitoids (Obermaier *et al.*, 2001), predators (Ikeda & Nakasuji, 2002), pathogens (Kellner, 2002), or abiotic factors (Madrid & Stewart, 1981; Smitley *et al.*, 1998). Oviposition site selection in general can be triggered by various factors, such as previous egg load of the host plant and future host plant supply (Mappes & Mäkelä, 1993; Nomakuchi *et al.*, 2001), future larval performance (Kouki, 1993), plant quality (Kouki, 1991), plant architecture (Gingras *et al.*, 2003), surrounding vegetation structure (Meiners & Obermaier, 2004), and microclimate (Kopper *et al.*, 2000).

Host plant height was examined in several studies as a factor potentially determining the choice of oviposition site or number of eggs laid (Hopkins & Whittaker, 1980; Björkman *et al.*, 1997; Nomakuchi *et al.*, 2001; Agrawal & van Zandt, 2003). However, oviposition height within the host plant was only rarely investigated (Leite *et al.*, 1999; Satoh, 2002). There are no studies at all on oviposition height *per se* that exclude the effect of host plant characteristics simply changing with plant height. The effect of oviposition height on egg survival due to effects of winter mortality (Smitley *et al.*, 1998) or parasitism (Wang *et al.*, 1997) and the resulting selection pressures responsible for spatial patterns of egg distribution are very rarely examined as well.

The polyphagous tansy leaf beetle, *Galeruca tanaceti* Linnaeus (Coleoptera: Chrysomelidae), can occur as pest on potato, cabbage, beans, dahlia and other cultural plants (Lühmann, 1939; Heinze, 1974). The females oviposit at different heights on dry stalks of grasses or herbs of mostly non-host plants during autumn (Prevett, 1953; Meiners & Obermaier, 2004). An egg clutch contains on average 64.9 ± 1.3 eggs (E. Obermaier, unpublished data). The species overwinters in the egg stage and egg clutches stay on the plants for up to 7 months until hatching of the larvae in April (Obermaier & Zwölfer, 1999). During this time, the eggs are exposed to numerous biotic and abiotic mortality factors. While the egg parasitoid *Oomyzus galerucivorus* Hedqvist (Hymenoptera: Eulophidae) parasitizes the eggs mainly from September to November, the eggs have to face detrimental abiotic factors, pathogen attack and predation throughout the whole winter. The effect of abiotic factors, pathogens and predation on egg survival can be conveniently described as winter mortality. We examined the variability of the oviposition height of *G. tanaceti* in space and time and proposed several hypotheses with ecological and evolutionary explanations for the observed patterns. A sub-set of these hypotheses was tested in the field. Specifically, we wanted to know whether oviposition height might be associated with vegetation height, egg parasitism or winter mortality. The following questions were asked: (i) Is there a standard uniform oviposition height for *G. tanaceti*, independent of the time of season and habitat? (ii) Is egg clutch mortality related to oviposition height: Does winter mortality of egg clutches change with oviposition height? Does egg parasitism change with the height of the oviposition site?

8.2 MATERIAL AND METHODS

8.2.1 Study system

The study was conducted in the nature conservation area Hohe Wann in Lower Franconia, Germany, in autumn and winter of 2001/2002 and 2005. Due to the high time requirement for conducting phenological studies, only three sites were investigated at the same time. The three sites chosen for investigation were similar in most aspects so that a comparison of the oviposition phenology between them seems to be justified. All three sites showed sufficiently high densities of ovipositing females, a moderate density of the main host plant, *Achillea millefolium* L. (Asterales, Asteraceae), comparable slopes, the same biotope type (semi-arid grassland), are located at approximately the same altitude (‘Prappach’: 300 m, ‘Schafhof’: 280 m, and ‘Rauchberg’: 343 m above NN) and are only 909-2190 m apart from each other. They differ, however, in their exposure. The site ‘Prappach’ has a north-west orientation, the sites ‘Schafhof’ and ‘Rauchberg’ are south oriented. The study was conducted on sub-squares (size 1100-2800 m^2) of all three sites. The largest site (‘Schafhof’) was additionally used to study egg parasitism and winter mortality of *G. tanaceti* on a small spatial scale. Additionally, eleven similar semi-arid grassland sites in the same study area were selected for an investigation of oviposition height and vegetation structure at different sites.

The leaf beetle *Galeruca tanaceti* is a univoltine species distributed throughout Europe and in the East Palaearctic region, Near East, and North Africa (Fauna Europaea Web Service, 2004; version 1.1, http://www.faunaeur.org). It is therefore exposed to a wide range of abiotic conditions ranging from environments which are snow-free throughout winter to ones which have a much more prolonged cover of snow (e.g. Sweden, Finland) than the study sites in southern Germany.

The egg parasitoid *Oomyzus galerucivorus* parasitizes egg clutches of its host shortly after oviposition of the host in autumn. The parasitoid larvae hibernate in the host eggs and adults emerge next spring. The 1.5 mm long egg parasitoids search for host egg clutches by walking up and down vertical structures within the vegetation (T. Meiners, unpublished data).

8.2.2 Phenology of oviposition height

On the three grassland sites selected the oviposition height of *G. tanaceti* was recorded over the oviposition season from September until December. Every 4 days the oviposition height of 18 egg clutches per site was measured as vertical height. All egg clutches at each date were marked, so that newly oviposited eggs could be distinguished. Only these were used for the investigations and a random sample of 18 egg clutches was also measured. With this method we also ensured a representative range of oviposition heights. We used the sample size of 18 egg clutches per date and per site because this was the largest estimated common sample size possible to collect on each of the three sites over a longer period of time. Since one female deposits only one egg clutch per week, it is usually difficult to find sites featuring enough newly oviposited egg clutches for permanent monitoring. By choosing an interval of four days to search for new laid egg clutches we were also able to investigate the current rate of parasitism at the same time. After mid-November newly laid egg clutches were searched for after longer time intervals, however, the collected eggs were 10 randomly selected squares within each site once during the oviposition period in October.

8.2.3 Oviposition and vegetation height at different field sites

On 11 dry grassland sites oviposition height was recorded for 20 randomly selected egg clutches per site. For each of the egg clutches the total height of the plant to which it was attached was also measured. Mean and maximal vegetation height was measured in fifteen 0.25 m^2 squares per site.

8.2.4 Parasitism and oviposition height

Selection of egg clutches – In October/November the influence of oviposition height on egg parasitism was investigated. For the study of egg parasitism 60 random points were created on an aerial photo of the grassland site via the spatial analyst module of the ArcView GIS software package (ESRI, Redlands, California). In the field, the random points were located with a GPS (Garmin). The egg clutch nearest to the obtained point was selected by the nearest neighbor method and marked with a stick. In all of the 60 randomly selected plant individuals, oviposition height was recorded as vertical height from the ground to the egg clutch. Afterwards, the egg clutches were harvested and transferred to the laboratory.

Hatching of parasitoids – All egg clutches collected from the site ($n = 60$) were put singly into vials, which were sealed with a very fine net and kept for several weeks at room temperature until parasitoids hatched. The net was sprayed every three days with water to prevent egg clutches from drying out. Parasitism was registered as incidence.

If at least one parasitoid had hatched from the clutch it was categorized as incidence (1), if no parasitoid had hatched it was a non-incidence (0).

Dissection of egg clutches – All eggs of the 60 egg clutches were additionally dissected in the laboratory to calculate the rate of parasitism per egg clutch. In order to achieve this, the number of parasitoids hatched, number of viable parasitoids within the egg shells, and total egg number were counted.

8.2.5 Winter mortality and oviposition height

In December, 135 plants with egg clutches of *G. tanaceti* were marked with yellow tape at the 'Schafhof' site. Egg clutches on herbs and grasses were tested in the same proportions. The tape was applied to a stalk next to the stalk with the egg clutch, to avoid any change in the stability of the plant. The height of the egg clutch was recorded as vertical height from the ground and as length of the stalk up to the egg clutch. Additionally plant type (grass/herb), number of ramifications and oviposition substrate (leaf, flower etc.) was recorded. Marked egg clutches were searched for again in April, after a long and deep cover of snow on the site during winter. In some cases it was difficult to relocate the markings because heavy snow cover had destroyed many plants and eggs and some marking indicators could not be found. Thus eggs clutches were searched for within a radius of 50 cm around the site of the tape mark for four minutes. After this time the egg clutch was categorized as either a) found on a plant or b) found on the ground or c) as disappeared (= destroyed).

All egg clutches were transferred to the laboratory. Five eggs per egg clutch were dissected. If a fully developed beetle larva had been found in at least one of the eggs we categorized the egg clutch as 'survived' (1). If the egg clutch had disappeared in the field or if a fully developed beetle larvae could not be found during dissection, the egg clutch was categorized as 'not survived' (0).

8.2.6 Statistics

The change in oviposition height of *G. tanaceti* over the season (12 dates in the space of time 10/2-12/1) on three different sites was tested with ANCOVA (Crawley, 2002). Differences between sites were tested by the site term. Differences between slopes of the regression lines by the date × site interactive term and differences of the slope from zero were tested by the date term. All single correlations were tested with the Pearson correlation coefficient, group differences between two groups were investigated with the paired *t*-test, after testing for normal distribution. The influence of oviposition height on parasitism and winter mortality of egg clutches was tested by multiple logistic regression (Jongman *et al.*, 1995; Hosmer & Lemeshow, 2000; see also Meiners & Obermaier, 2004). Models were accepted only if forward and backward logistic regression methods resulted in the same combination of habitat variables. In order to evaluate the goodness-of-fit of the habitat models the coefficient of determination R^2 after Nagelkerke (1991) was considered. Influence of oviposition height on rate of parasitism per egg clutch was tested by multiple regression. All procedures were calculated with the software package SPSS 10.0 (SPSS Inc., 2001).

8.3 RESULTS

8.3.1 Phenology of oviposition height

Oviposition height was recorded on three sites over the whole oviposition period (Figure 8.1). Oviposition height of the three sites differed significantly ($F_{site} = 937.61$, $P = 0.001$, $n = 18$). The slopes of the regression lines of all sites also differed significantly from zero ($F_{date} = 124.759$, $P = 0.001$, $n = 18$) and oviposition height declined over the season ($B_{date} = -2.192$, $SE = 0.196$, $t = -11.170$, $P = 0.001$ $n = 18$) with the same slope for all three sites, since the site-by-date interaction was not significant ($F_{site \times date} = 0.920$, $P = 0.399$ $n = 18$). The three studied sites differed in mean vegetation height (mean height ± SD): 'Rauchberg': 0.31 m ± 0.21 m, 'Schafhof': 0.31 m ± 0.17 m, 'Prappach': 0.59 m ± 0.25 m. The un-grazed 'Prappach' site with the highest mean vegetation height also showed the highest oviposition height.

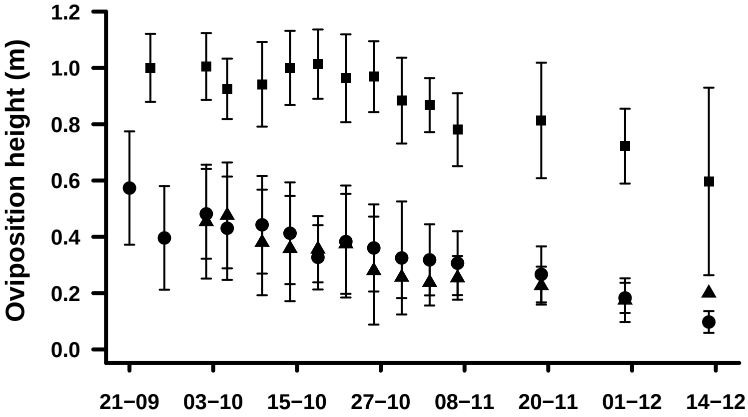

Figure 8.1: Development of the oviposition height during the oviposition period of *Galeruca tanaceti* at three sites in autumn ($R_{corrected} = 0.779$, $F_{date} = 124.759$, $P_{date} = 0.001$, $F_{site} = 937.610$, $P_{site} = 0.001$, $n = 18$). Only eggs newly deposited up to each date were used for the calculation. Sites examined were Prappach (square), Rauchberg (triangle) and Schafhof (circle). Mean values and standard deviation are given.

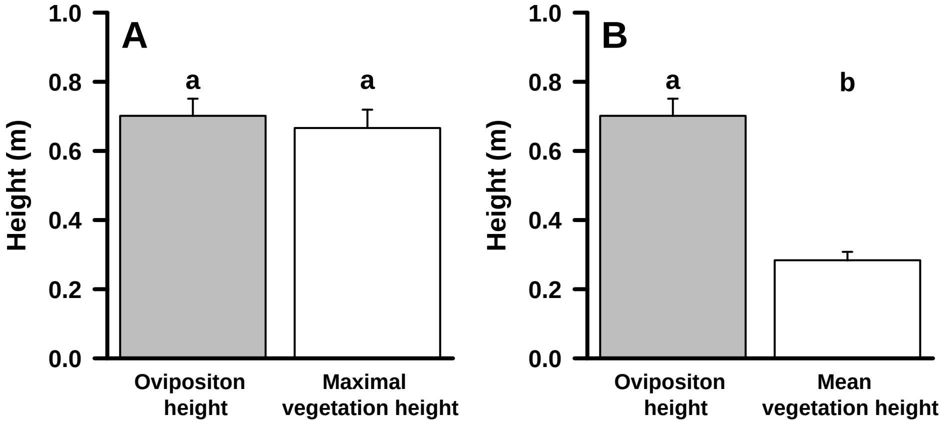

Figure 8.2: (A) Mean oviposition height of *Galeruca tanaceti versus* mean vegetation height at different sites ($t = 9.65$, $P < 0.001$, $n = 11$). (B) Mean oviposition height *versus* maximal vegetation height at different sites ($t = 0.794$, $P = n.s.$, $n = 11$). Different lower case letters indicate significantly different means. Illustrated are mean and standard error.

8.3.2 Oviposition height in different habitats

There was a significantly positive correlation between mean oviposition height and mean maximal vegetation height for 11 dry grassland sites ($r_p = 0.641$, $P = 0.034$, $n = 11$). There was no significant correlation of mean oviposition height and mean vegetation height ($r_p = 0.493$, $P = 0.123$, $n = 11$). Oviposition height seemed to increase with increasing maximal vegetation height of sites. Additionally, mean oviposition height was significantly higher than mean vegetation height ($t = 9.65$, $P < 0.001$, $n = 11$), but did not differ significantly from mean maximal vegetation height ($t = 0.794$, $P = n.s.$, $n = 11$) (Figure 8.2).

Oviposition height and height of plants on which egg clutches were attached were significantly positively correlated ($r_p = 0.934$, $P < 0.001$, $n = 220$) (Figure 8.3). This implies that the higher the plants used for oviposition, the higher the oviposition site on the selected plants.

8.3.3 Parasitism of egg clutches at different heights

The probability of an egg clutch being parasitized decreased significantly with increasing vertical height of the oviposition site above ground ($R^2_{Nagelkerke} = 0.095$, $P_{model} = 0.036$, coefficient $= -0.027$, $SE = 0.014$, $P_{variable} = 0.047$, $n = 60$) (Figure 8.4). When the egg clutches were dissected for total rate of parasitism per egg clutch, results were similar. Rate of parasitism per egg clutch was significantly negatively correlated with oviposition height ($R^2_{corrected} = 0.051$, $F = 4.070$, $P_{model} = 0.048$, coefficient

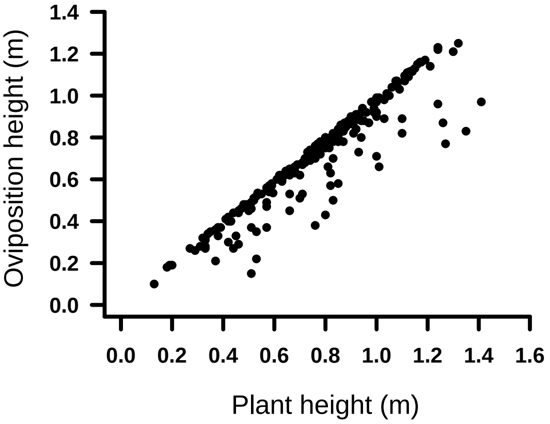

Figure 8.3: Correlation of oviposition height with height of plants to which the egg clutches were attached to ($r_p = 0.934$, $P < 0.001$, $n = 220$).

$= -0.006$, $SE = 0.003$, $P_{variable} = 0.048$, $n = 60$).

8.3.4 Winter mortality of egg clutches at different heights

Of 135 markings attached to the stalks of egg clutches in autumn, 85 markings and 21 egg clutches belonging to the markings were found again in spring. Of the 21 egg masses successfully recovered, 13 were found on the ground and 8 on plants. Five of 13 egg clutches found on the ground were no longer viable, as was 1 of 8

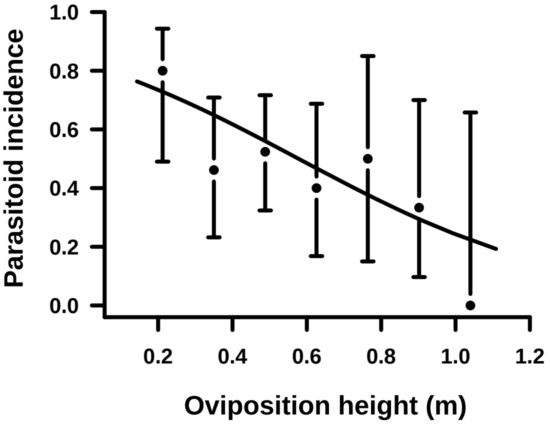

Figure 8.4: Parasitism of the egg clutches of the leaf beetle *Galeruca tanaceti* by the parasitoid *Oomyzus galerucivorus* at different oviposition heights. Plotted are the mean ± 95 % CI of parasitoid incidences in oviposition height classes of 0.14 m (circles with bars) and the predicted probability of parasitoid incidence (line) calculated by the logistic regression model (n = 60 egg clutches).

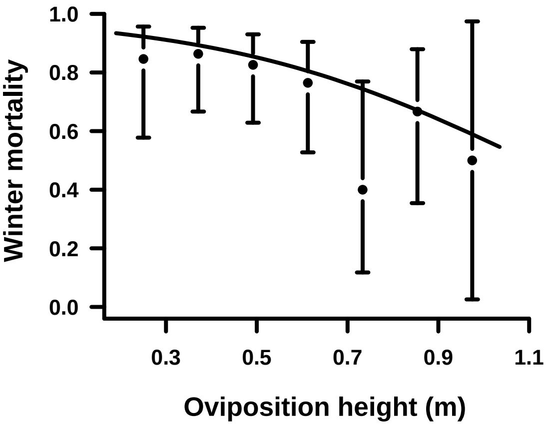

Figure 8.5: Winter mortality of the egg clutches of the leaf beetle *G. tanaceti* at different oviposition heights. Plotted are the mean ± 95 % CI of the incidences of winter mortality (summarizing mortality by pathogens and abiotic factors) in oviposition height classes of 0.12 m (circles with bars) and the predicted probability of the incidence of winter mortality (line) calculated by the logistic regression model ($n = 85$ egg clutches).

egg clutches found on plant structures. Winter egg clutch mortality decreased significantly with increasing vertical oviposition height above ground (winter mortality of egg clutches: $R^2_{Nagelkerke} = 0.77$, $P_{model} = 0.047$, coefficient $= -0.029$, $SE = 0.015$, $P_{variable} = 0.049$, $n = 85$) (Figure 8.5).

Oviposition height was the only factor which significantly could explain winter mortality. The other variables tested, plant type (grass/herb), number of first order ramifications and oviposition substrate (leaf, flower, etc.) did not contribute to the model.

8.4 DISCUSSION

Oviposition height of *G. tanaceti* decreased significantly over the season and varied strongly over a range of sites examined. This suggests, in contrast to the observations of Scherf (1966), that this beetle species does not prefer a uniform standard oviposition height but that this parameter is extremely variable in time and space. Oviposition height seems to depend proximately on the vegetation height of the sites and the height of the individual plants selected for oviposition. The climatic conditions during the season, as well as the physiological state of the females, might further contribute to the actual height.

Oviposition height was highest at the beginning of the oviposition period and decreased later significantly to 80-40 % of the initial height on all three sites examined. This could be interpreted as a declining ability of the gravid female beetles to climb high up the stalks. However, a comparison of the oviposition height at the different sites contradicts this hypothesis since at the end of the season at the site ‘Prappach’ females oviposited as high as females at the other two sites at the beginning of the oviposition season in September. On the other hand, over the course of the oviposition period (autumn), the availability of high plant structures, attractive for oviposition by *G. tanaceti*, might decline because high structures at suitable places are either already occupied with previous egg clutches or become rarer by natural degradation during autumn (B. Randlkofer, personal observations).

The comparison of oviposition height and vegetation height at different sites supported the second interpretation and revealed a significantly positive correlation of oviposition height and maximal vegetation height. Furthermore, mean height of egg clutches was significantly higher than mean vegetation height, and ranged at the height of the mean maximal vegetation height. Oviposition height was also significantly positively correlated with the height of the plants to which egg clutches had been attached. These results suggest that female beetles select oviposition sites high above the mean vegetation height and try to oviposit as high as possible within the vegetation and on the selected plants. Beside the proximate factors which seem to influence oviposition height, such as available vegetation height, we wanted to investigate hypotheses on main selection pressures which might have induced herbivores to oviposit high up in herbaceous vegetation. Parasitism by the specialized egg parasitoid *Oomyzus galerucivorus* (the only egg parasitoid of *G. tanaceti* in this region) and mortality by abiotic factors, pathogens or removal by predators during winter, summarized as winter mortality, were examined as the main mortality factors of the egg stage of the tansy leaf beetle. Both mortality factors were significantly reduced with increasing oviposition height above ground in the present study.

Parasitism significantly decreased with increasing oviposition height. As the egg parasitoid mainly searches for its host by running up vertical structures (T. Meiners, personal observations), ovipositing high up in the vegetation seems to help to prevent the higher located egg clutches from being parasitized. It might therefore represent a selection advantage for these eggs. Egg-parasitism of the European corn borer also was lower in the upper parts of corn plants (Wang *et al.*, 1997). Gingras *et al.* (2002, 2003) showed that plant structure affected the host finding success of parasitoids which was higher on plants with a simple structure and low on plants with a complex structure. On certain microsites of plants mortality was reduced (Berdegué *et al.*, 1996; Hopkins & Dixon, 1997). According to the results of our study oviposition sites located high up in the vegetation might represent an enemy-free space as well.

Like parasitism, winter mortality in *G. tanaceti* eggs was lower when the oviposition site was located high up in the vegetation. It can therefore explain the preference for high oviposition sites by *G. tanaceti*. The result stands in contrast to the findings of two studies on winter mortality of eggs of the gypsy moth in the USA and Canada (Madrid & Stewart, 1981; Smitley *et al.*, 1998). There, due to the snow cover, eggs close to the ground have a higher chance of survival than eggs high above the snow. We, at present, are not able to solve the question as to why there is a higher survival from winter mortality factors at higher oviposition sites with currently available data. Field observations show that most stalks are still upright after the first heavy snow falls at the beginning of January (E. Obermaier, personal observations) and that original oviposition height therefore might still influence survival during the ongoing winter. Hypotheses for a higher survival of eggs laid higher in the vegetation include a possibly lower chance of deterioration of those egg clutches and higher temperatures high above the ground. Previously it had been shown that *G. tanaceti* prefers a sunny microclimate and therefore higher temperatures for oviposition (Meiners & Obermaier, 2004).

Winter egg mortality in herbivorous insects can be caused by pathogens such as fungi (Fargues & Rodriguez, 1980; Storey *et al.*, 1991; Long *et al.*, 1998; Tallamy *et al.*, 1998), or bacteria (Robert *et al.*, 1998), or by removal due to predators. The interaction of pathogens and abiotic factors (such as wind, heavy rain or snow) might enhance the deterioration and disappearance of most of the egg clutches fallen to the ground over the winter. Our data suggest that a significantly higher proportion of egg clutches which remained on the stalks over the winter were viable, compared with those that had fallen to the ground ($\chi^2 = 14.09$, df $= 1$, $P > 0.001$, $n = 21$). Egg clutches found to be fed upon by arthropod predators in autumn were very rare (about 5 % of the egg clutches were preyed upon in a recent investigation, E. Obermaier, unpublished data). Vertebrate predators, like birds, might also contribute to the high number of egg clutches disappearing completely from the ground. However, birds were shown to avoid the larvae of *Galeruca tanaceti* because they contain anthraquinones as a defence agent (Hilker & Köpf, 1994). Since not only larvae, but also the eggs of *G. tanceti* contain anthraquinones, they might be protected against vertebrate predators as well (Meiners *et al.*, 1997).

Oviposition height in *G. tanaceti* is variable in time and space and probably dependent on vegetation height. The females seem to oviposit as high as possible in the herbaceous vegetation. Mortality caused by the main mortality factors of the eggs, ‘egg parasitism’ and ‘winter mortality’, summarizing all mortality factors acting upon the egg clutches over the winter, is dependent on oviposition height and is significantly reduced with increasing oviposition height. High oviposition sites of *G. tanaceti* can therefore be explained by and have possibly evolved through reduced mortality, and may thus represent an enemy-free space.

Summary

Herbivorous insects are the major link between the primary producers and a multitude of animals at higher trophic levels. Thus, elucidating the causes and consequences of their distribution patterns within the ‘green world’ is essential for our understanding of numerous ecological processes on multiple spatial scales. We can ask where and why a certain herbivore is found in the landscape, inside the habitat, on which plants within the habitat, and finally, on the plant. Depending on spatial scale, different processes (fitness considerations, physiological abilities, population dynamics, dispersal behavior, the history of the landscape, etc.) shape the distribution of herbivores.

Scaling down from fragmented landscapes to individual host plants, this thesis analyzes the distribution patterns of the strictly monophagous tortoise beetle *Cassida canaliculata* Laich. (Coleoptera: Chrysomelidae), which feeds and oviposits exclusively on meadow sage, *Salvia pratensis* L. (Lamiales: Lamiaceae), and compares them to those of the polyphagous tansy leaf beetle *Galeruca tanaceti* L. (Coleoptera: Chrysomelidae), which does not oviposit on host plants but on dry non-host structures.

The first manuscript (Chapter 3) describes the distribution pattern of *C. canaliculata* within the fragmented landscape of the nature reserve ‘Hohe Wann’. Habitats that were used for oviposition were large, well-connected, and of high habitat quality. While the occurrence probability of *C. canaliculata* was dominantly influenced by patch size and isolation, the population density of the beetles was affected most by host plant density. The occurrence probability increased with increasing patch size and decreasing isolation. The population density increased with increasing host plant density. Based on metapopulation theory, large, well-connected patches have a higher chance of being found by dispersing animals and large and high-quality patches have a reduced risk of extinction. In the present study, habitat quality was not correlated to patch size. However, habitat quality influenced the population density of *C. canaliculata* more strongly than patch size. This result stresses the importance of habitat quality as the ‘missing third parameter’ in metapopulation dynamics, as also smaller patches of high host plant density may support viable populations of *C. canaliculata*. Chapter 3 further investigates the distribution pattern of *C. canaliculata*’s egg parasitoid *Foersterella reptans* Nees (Hymenoptera: Tetracampidae). The occurrence probabil-

ity of the parasitoid increased only with increasing population density of the beetle, whereas none of the other studied parameters was correlated with parasitoid occurrence or density.

The distribution of the generalist tansy leaf beetle *G. tanaceti* and its egg parasitoid *Oomyzus galerucivorus* Hedqvist (Hymenoptera: Eulophidae) within the nature reserve 'Hohe Wann' was correlated to the vegetation structure of habitat patches (Chapter 7). Both beetle and parasitoid were found more often on patches with little shrub cover. Moreover, the occurrence probability of *O. galerucivorus* also increased with increasing population density of the tansy leaf beetle. As Chapter 7 focuses on the influence of vegetation structure, the importance of patch size, isolation, and habitat quality was not or only partially investigated and can thus not be compared to the results on *C. canaliculata*.

The question, where *C. canaliculata* can be found within the habitat was already investigated within the scope of a previous study (Heisswolf *et al.*, in press). *C. canaliculata* preferred microhabitats with a high percentage cover of its single host plant *S. pratensis*, whereas other microhabitat parameters like vegetation structure did not influence the occurrence probability of the beetle. Moreover, the probability of parasitism by *F. reptans* was not related to any of the studied microhabitat characteristics, i.e. *C. canaliculata* seemed to choose microhabitats for oviposition based on host plant availability and not on enemy-free space.

In contrast, the microhabitat choice of the generalist tansy leaf beetle *G. tanaceti* was strongly negatively correlated with the risk of parasitism, i.e. the beetles preferred to oviposit in microhabitats with dense vegetation, while the probability of parasitism decreased with increasing density of the vegetation (Chapter 7). Thus, for *G. tanaceti* microhabitats with a dense vegetation seemed to provide enemy-free space. Still, the availability of host plants within the microhabitat – which was not investigated in the present study – is also expected to be of central importance to *G. tanaceti*.

The next two manuscripts (Chapter 4 & Chapter 5) deal with the process of host finding and host recognition in *C. canaliculata*. Laboratory experiments showed that the beetles recognize their host plant *S. pratensis* chiefly by qualitative rather than quantitative contact stimuli, while olfactory cues (i.e. host plant recognition from a distance) seem to play only a minor role (Chapter 4). A fractionation of the behaviorally active host plant extracts revealed that at least two distinct non-polar contact stimuli are needed for host plant identification. Furthermore, explorative experiments on the relevance of visual cues for *C. canaliculata* showed that the beetles are able to respond to optical signals, however, they could not elucidate whether vision also plays a role in host plant recognition.

In addition to the laboratory experiments, a semi-natural arena was developed, in which the movement patterns of *C. canaliculata* were examined depending on whether a host (*S. pratensis*), a non-host (*Rumex* sp., Caryophyllales: Polygonaceae), or no plant was presented in the center of the arena (Chapter 5). While the movement pattern analysis yielded no differences in the ‘general’ walking parameters track length, straightness, and walking speed, the beetles walked closer to the center and oriented more towards the center of the arena when a host plant was presented there. Contrary to Chapter 4, these results show that *C. canaliculata* is able to recognize its host plant *S. pratensis* from a distance when a ‘real’ plant is offered. The fact that the growth form of the host *S. pratensis* and the non-host *Rumex* sp. is very similar (at least to the human eye) suggests that in this more natural setting, olfactory cues (or a combination of olfactory and visual cues, e.g. plant color) may well be used for host plant finding. A further result of this study corroborates the finding of Chapter 4 that *C. canaliculata* is able to recognize *S. pratensis* by contact stimuli, as none of the beetles that reached the host plant ever left it again, while two of three beetles left the non host.

Regarding the question, which individual plants are used for oviposition by *C. canaliculata*, a previous study by Heisswolf *et al.* (in press) revealed that the females seemed to prefer large host plants. Chapter 6 now analyzes the fitness consequences of growing up on large (r > 20 cm) or small (r < 10 cm) host plant individuals for larvae of *C. canaliculata* both in laboratory and in field experiments. In the laboratory experiment, larvae developed faster on leaves of large host plants, which may be of selective advantage in the field. Chemical analyses further showed that leaves of large plants had a significantly higher leaf nitrogen content, which most probably facilitated faster larval development. The predator-exclusion experiment in the nature reserve ‘Hohe Wann’ could not validate this difference in development time, which probably resulted from more variable climatic conditions in the field. However, larvae had a higher survival probability on large host plants when all predators had free access to the plants. This may be due to the more complex architecture of these plants, which could hamper the searching efficiency of natural enemies. The results of this study emphasize the importance of experiments under natural conditions, which often yield results different from these obtained in laboratory studies. Nevertheless, both laboratory and field experiments provided evidence for a selective advantage of ovipositing on large individuals of *S. pratensis*.

The polyphagous tansy leaf beetle *G. tanaceti* does – as already mentioned above – not oviposit on host plants but on dry non-host structures, mostly on grass stalks. Thus, the question of oviposition site choice on the plant scale cannot be analyzed regarding plant quality as in *C. canaliculata*. Still, as the dry plant stalks differ in their structural complexity, beetles may choose plants for oviposition based on structural plant features. However, Chapter 7 shows that – although eggs of *G. tanaceti* were more often found on simply structured plants – the beetles seemed to distribute their eggs according to the availability of these structures within the habitat.

Finally, the question remains, where a herbivore should oviposit on a plant. This issue was not analyzed for *C. canaliculata*, but only for *G. tanaceti*. Chapter 8 describes the correlations between oviposition height, winter mortality and egg parasitism in *G. tanaceti*. Choosing oviposition sites as high as possible in the vegetation seemed to be of selective advantage to the beetles, as the risk of both winter mortality and parasitism decreased with increasing oviposition height.

The results of this thesis show that the distribution pattern of the specialized herbivore *Cassida canaliculata* was predominantly influenced by the distribution and quality of its single host plant species *Salvia pratensis*, while enemy-free space seemed to influence oviposition site choice only regarding larval performance on the scale of individual host plants. In contrast, the distribution pattern of the generalist herbivore *Galeruca tanaceti* was shaped by interactions with its egg parasitoid both regarding microhabitat choice and distribution of egg clutches within individual plants. Thus, the degree of specificity of a herbivore seems to be of primary importance to the resulting distribution pattern on all spatial scales.

Zusammenfassung

Herbivore Insekten sind das zentrale Bindeglied zwischen den Primärproduzenten und einer Vielzahl von Tieren höherer trophischer Ebenen. Daher ist es für unser Verständnis von unzähligen ökologischen Prozessen auf multiplen räumlichen Skalen essentiell, die Ursachen und Folgen ihrer Verteilungsmuster in der "grünen Welt" aufzuklären. Wir können fragen, wo und warum ein bestimmter Herbivor in der Landschaft, im Habitat, auf welcher Pflanze im Habitat und schließlich wo auf dieser Pflanze zu finden ist. In Abhängigkeit von der räumlichen Skala wird die Verteilung der Herbivoren von unterschiedlichen Prozessen (Fitness-Überlegungen, physiologische Fähigkeiten, Populationsdynamik, Dispersalverhalten, Geschichte der Landschaft etc.) geformt.

Herunterskalierend von fragmentierten Landschaften zu individuellen Wirtspflanzen, untersucht diese Doktorarbeit die Verteilungsmuster des streng monophagen Blattkäfers *Cassida canaliculata* Laich. (Coleoptera: Chrysomelidae), der ausschließlich auf Wiesensalbei, *Salvia pratensis* L. (Lamiales: Lamiaceae), frißt und Eier ablegt, und vergleicht sie mit denen des polyphagen Rainfarnblattkäfers *Galeruca tanaceti* L. (Coleoptera: Chrysomelidae), der seine Eier nicht auf Wirtspflanzen, sondern auf trockenen nicht-Wirtsstrukturen ablegt.

Das erste Manuskript (Kapitel 3) beschreibt das Verteilungsmuster von *C. canaliculata* in der fragmentierten Landschaft des Naturschutzgebiets "Hohe Wann". Zur Eiablage wurden große, gut verbundene Habitate von hoher Habitatqualität genutzt. Während die Vorkommenswahrscheinlichkeit von *C. canaliculata* am stärksten von der Patchgröße und -isolation beeinflußt wurde, war die Populationsdichte am meisten von der Habitatqualität abhängig. Die Vorkommenswahrscheinlichkeit nahm mit steigender Patchgröße und mit abnehmender Patchisolation zu. Die Populationsdichte stieg mit zunehmender Wirtspflanzendichte. Basierend auf der Metapopulationstheorie haben große, gut verbundene Patches eine höhere Chance, von dispergierenden Tieren gefunden zu werden und große Patches von hoher Habitatqualität haben ein reduziertes Extinktionsrisiko. In der vorliegenden Studie war die Habitatqualität nicht mit der Patchgröße korreliert. Aber die Habitatqualität hatte einen stärkeren Einfluß auf die Populationsdichte von *C. canaliculata* als die Patchgröße. Dieses

Ergebnis betont die Bedeutung der Habitatqualität als "fehlender dritter Parameter" in Metapopulationsdynamiken, da auch kleinere Patches mit hoher Wirtspflanzendichte überlebensfähige Populationen von *C. canaliculata* aufrechterhalten können. Kapitel 3 untersucht darüber hinaus das Verteilungsmuster des Eiparasitoiden von *C. canaliculata*, *Foersterella reptans* Nees (Hymenoptera: Tetracampidae). Die Vorkommenswahrscheinlichkeit des Parasitoiden nahm nur mit steigender Populationsdichte des Käfers zu, wohingegen keiner der anderen untersuchten Parameter mit der Vorkommenswahrscheinlichkeit oder der Dichte des Parasitoiden korreliert war.

Die Verteilung des unspezialisierten Rainfarnblattkäfers *G. tanaceti* und seines Eiparasitoiden *Oomyzus galerucivorus* Hedqvist (Hymenoptera: Eulophidae) im Naturschutzgebiet "Hohe Wann" war mit der Vegetationsstruktur der Habitatpatches korreliert (Kapitel 7). Sowohl der Käfer als auch der Parasitoid waren häufiger auf Patches mit geringem Verbuschungsgrad zu finden. Darüber hinaus stieg die Vorkommenswahrscheinlichkeit von *O. galerucivorus* auch mit zunehmender Populationsdichte des Rainfarnblattkäfers. Da Kapitel 7 auf den Einfluß der Vegetationsstruktur fokussiert, wurde die Bedeutung von Patchgröße, Isolation und Habitatqualität nicht oder nur teilweise untersucht und kann daher nicht mit den Ergebnissen von *C. canaliculata* verglichen werden.

Die Frage, wo *C. canaliculata* im Habitat gefunden werden kann, wurde bereits im Rahmen einer früheren Studie untersucht (Heisswolf *et al.*, in press). *C. canaliculata* bevorzugte Mikrohabitate mit einer hohen prozentualen Deckung seiner einzigen Wirtspflanze *S. pratensis*, wohingegen andere Habitatparameter wie die Vegetationsstruktur die Vorkommenswahrscheinlichkeit des Käfers nicht beeinflußten. Darüber hinaus war die Parasitierungswahrscheinlichkeit durch *F. reptans* mit keiner der untersuchten Mikrohabitatparameter korreliert, d.h. *C. canaliculata* schien Mikrohabitate zur Eiablage basierend auf der Wirtspflanzenverfügbarkeit und nicht auf Feind-freiem Raum auszusuchen.

Im Gegensatz dazu war die Mikrohabitat-Wahl des unspezialisierten Rainfarnblattkäfers *G. tanaceti* stark negativ mit dem Parasitierungsrisiko korreliert, d.h. die Käfer bevorzugten Mikrohabitate mit dichter Vegetation zur Eiablage, wobei die Parasitierungswahrscheinlichkeit mit zunehmender Vegetationsdichte abnahm (Kapitel 7). Daher schienen Mikrohabitate mit einer dichten Vegetation Feind-freien Raum für *G. tanaceti* zu bieten. Nichtsdestoweniger spielt die Wirtspflanzenverfügbarkeit im Mikrohabitat – die im Rahmen dieser Studie nicht untersucht wurde – höchstwahrscheinlich ebenfalls eine zentrale Rolle für *G. tanaceti*.

Die nächsten beiden Manuskripte (Kapitel 4 & Kapitel 5) beschäftigen sich mit dem Prozeß der Wirtsfindung und -erkennung bei *C. canaliculata*. Laborexperimente zeigten, daß die Käfer ihre Wirtspflanze hauptsächlich durch qualitative statt durch quantitative Kontaktreize erkennen, während olfaktorische Reize (d.h. Wirtspflanzenerkennung aus der Distanz) nur eine untergeordnete Rolle zu spielen scheinen (Kapitel 4). Eine Fraktionierung der verhaltensaktiven Wirtspflanzenextrakte ergab, daß mindestens zwei unterschiedliche unpolare Kontaktreize zur Wirtspflanzenidentifizierung notwendig sind. Darüber hinaus zeigten explorative Experimente zur Relevanz optischer Reize für *C. canaliculata*, daß die Käfer in der Lage sind, auf optische Reize zu reagieren, sie konnten jedoch nicht aufklären, ob das Sehen auch eine Rolle bei der Wirtspflanzenerkennung spielt.

Zusätzlich zu den Laborexperimenten wurde eine halbnatürliche Arena entwickelt, in der die Laufmuster von *C. canaliculata* in Abhängigkeit davon untersucht wurden, ob eine Wirtspflanze (*S. pratensis*), eine nicht-Wirtspflanze (*Rumex* sp., Caryophyllales: Polygonaceae), oder überhaupt keine Pflanze in der Mitte der Arena präsentiert wurde (Kapitel 5). Während die Laufmusteranalyse keine Unterschiede in den "allgemeinen" Laufparametern Spurlänge, Geradlinigkeit und Laufgeschwindigkeit ergab, liefen die Käfer näher an der Arenamitte und orientierten sich mehr zur Arenamitte wenn dort eine Wirtspflanze angeboten wurde. Im Gegensatz zu Kapitel 4 zeigen diese Ergebnisse, daß *C. canaliculata* in der Lage ist, seine Wirtspflanze *S. pratensis* aus der Distanz zu erkennen, wenn eine "reale" Pflanze angeboten wird. Die Tatsache, daß die Wuchsform der Wirtspflanze *S. pratensis* und der nicht-Wirtspflanze *Rumex* sp. ziemlich ähnlich ist (zumindest für das menschliche Auge) legt nahe, daß in diesem natürlicheren Versuchsaufbau olfaktorische Reize (oder eine Kombination aus olfaktorischen und visuellen Reizen, z.B. der Pflanzenfarbe) sehr wohl zur Wirtspflanzenfindung genutzt werden können. Ein weiteres Ergebnis dieser Studie bekräftigt den Befund von Kapitel 4, daß *C. canaliculata* in der Lage ist, *S. pratensis* anhand von Kontaktreizen zu erkennen, da keiner der Käfer, der die Wirtspflanze erreicht hatte, sie je wieder verlassen hat, wohingegen zwei von drei Käfern die nicht-Wirtspflanze verließen.

Bezüglich der Frage, welche Wirtspflanzenindividuen von *C. canaliculata* zur Eiablage genutzt werden, zeigte eine frühere Studie von Heisswolf *et al.* (in press), daß die Weibchen große Wirtspflanzen zu bevorzugen schienen. Kapitel 6 analysiert nun sowohl in Labor- als auch in Freilandexperimenten die Fitnesskonsequenzen für Larven, die auf großen (r > 20 cm) und kleinen (r < 10 cm) Wirtspflanzen aufwachsen. Im Laborexperiment entwickelten sich die Larven auf Blättern großer Wirtspflanzenindividuen schneller, was im Feld einen Selektionsvorteil darstellen könnte. Chemische Analysen zeigten außerdem, daß die Blätter großer Wirtspflanzen einen signifikant höheren Stickstoffgehalt aufwiesen, was höchstwahrscheinlich zu dem schnelleren Larvenwachstum geführt hat. Das Räuber-Ausschluß-Experiment im Naturschutzgebiet "Hohe Wann" konnte diesen Unterschied in der Entwicklungszeit nicht validieren, was höchstwahrscheinlich durch die variableren klimatischen Bedingungen im Feld verursacht wurde. Jedoch hatten die Larven eine höhere Überlebenswahrscheinlichkeit auf großen Pflanzen, wenn alle Räuber freien Zugang zu den Pflanzen hatten. Dies könnte durch die komplexere Architektur dieser Pflanzen bedingt sein, die die Sucheffizienz der natürlichen Feinde beeinträchtigen könnte. Die Ergebnisse dieser Studie betonen die Bedeutung von Experimenten unter natürlichen Bedingungen, deren Resultate sich oft von denen unterscheiden, die in Laborexperimenten erhalten wurden. Nichtsdestoweniger erbrachten sowohl die Labor- als auch die Freilandexperimente den Nachweis für einen selektiven Vorteil der Eiablage auf großen Individuen von *S. pratensis*.

Der polyphage Rainfarnblattkäfer *G. tanaceti* legt – wie bereits oben erwähnt – nicht auf Wirtspflanzen ab, sondern auf trockene nicht-Wirtsstrukturen, hauptsächlich auf Grashalme. Daher kann die Frage nach der Wahl des Eiablageplatzes auf der Pflanzenskala nicht wie bei *C. canaliculata* bezüglich der Wirtspflanzenqualität untersucht werden. Da sich die trockenen Pflanzenhalme jedoch in ihrer strukturellen Komplexität unterscheiden, könnten die Käfer Pflanzen basierend auf strukturellen Eigenschaften zur Eiablage auswählen. Jedoch zeigt Kapitel 7, daß die Käfer – obwohl die Eigelege von *G. tanaceti* häufiger auf einfach strukturierten Pflanzen gefunden wurden – ihre Gelege gemäß der Verfügbarkeit dieser Strukturen im Habitat zu verteilen schienen.

Schließlich bleibt die Frage, wo ein Herbivor auf der Pflanze ablegen sollte. Diese Frage wurde nicht für *C. canaliculata* sondern nur für *G. tanaceti* untersucht. Kapitel 8 beschreibt die Korrelationen zwischen der Eiablagehöhe, der Wintermortalität und der Eiparasitierung bei *G. tanaceti*. Eiablageplätze möglichst hoch in der Vegetation zu wählen, scheint den Käfern einen Selektionsvorteil zu bieten, da sowohl das Risiko der Wintermortalität als auch das der Parasitierung mit zunehmender Eiablagehöhe abnahm.

Die Ergebnisse dieser Doktorarbeit zeigen, daß das Verteilungsmuster des spezialisierten Herbivoren *Cassida canaliculata* hauptsächlich von der Verteilung und Qualität seiner einzelnen Wirtspflanzenart *Salvia pratensis* abhing, während Feind-freier Raum die Wahl des Eiablageplatzes nur bezüglich der Larvalentwicklung auf der Skala des Wirtspflanzenindividuums zu beeinflussen schien. Im Gegensatz dazu wurde das Verteilungsmuster des unspezialisierten Herbivoren *G. tanaceti* sowohl bezüglich der Wahl des Mikrohabitats als auch der Verteilung der Eigelege innerhalb individueller Pflanzen durch die Interaktionen mit seinem Eiparasitoiden geformt. Daher scheint der Spezifizierungsgrad eines Herbivoren für das resultierende Verteilungsmuster auf allen räumlichen Skalen von zentraler Bedeutung zu sein.

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Conferences & Workshops

Visited Conferences

Conference of the *Gesellschaft für Ökologie (GFÖ)* 2002, Cottbus, Germany – talk

Conference of the *Deutsche Gesellschaft für allgemeine und angewandte Entomologie (DGaaE)* 2003, Halle/Saale, Germany – poster

Conference of the *Gesellschaft für Ökologie (GFÖ)* 2003, Halle/Saale, Germany – talk

Conference of the *DZG (Deutsche Zoologische Gesellschaft)* study group *'Evolutionary Biology'* 2004, Münster, Germany

Conference of the *Gesellschaft für Ökologie (GFÖ)* 2004, Gießen, Germany

Conference of the *Deutsche Gesellschaft für allgemeine und angewandte Entomologie (DGaaE)* 2005, Dresden, Germany – talk

Visited Workshops

'Simulations in ecology' 2002, Pesina, Italy

'Large scale dispersal of bush crickets' 2003, Fabrikschleichach, Germany

'Habitat suitability models' 2003, Leipzig, Germany

'Multitrophic interactions' 2004, Göttingen, Germany – talk

Curriculum Vitae

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EDUCATION

1985 – 1998	Primary school Esselbach & secondary school Balthasar-Neumann-Gymnasium Marktheidenfeld
1998 – 2003	University studies in biology (diploma) at the 'Bayerische Julius-Maximilians-Universität Würzburg'
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FOREIGN EXPERIENCE

March 2005	Participation in a two-week PhD-Course 'Insect Chemical Ecology' at the SLU (Swedish University of Agricultural Sciences) in Alnarp, Sweden

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Erklärung

gemäß § 4 Abs. 3 Ziff. 3, 5 und 8
der Promotionsordnung der Fakultät für Biologie der
Bayerischen Julius-Maximilians-Universität Würzburg

Hiermit erkläre ich ehrenwörtlich, die vorliegende Arbeit in allen Teilen selbständig und nur mit den angegebenen Quellen und Hilfsmitteln angefertigt zu haben.

Diese Dissertation hat weder in gleicher noch in ähnlicher Form in einem anderen Prüfungsverfahren vorgelegen.

Des Weiteren erkläre ich, daß ich früher weder akademische Grade erworben habe, noch zu erwerben versucht habe.

Würzburg, den 12. Mai 2006

(Annette Heißwolf)