Generalist insect herbivore performance in dependence of plant species richness, composition and quality

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Tag der Verteidigung: ……………………………………

Für meine Familie

Schön, dass ihr da seid

Manuscript overview and author contribution

Chapter I: "Plant species richness in montane grasslands affects the fitness of a generalist grasshopper species"

Sybille B. Unsicker, Alexandra Franzke, Juliane Specht, Günter Köhler, Jeanine Linz, Carsten Renker, Claudia Stein & Wolfgang W. Weisser

This observational field study was conducted to analyze the fecundity, expressed as oviposition rate and offspring number, of *Chorthippus parallelus* between 15 different meadow habitats in the study area. To discover the influence of plant species richness as well as of other habitat variables on the performance of a generalist insect herbivore was the main aim of this study.

Sybille B. Unsicker planned and performed the experiment, analyzed the data and wrote the manuscript. Alexandra Franzke provided data for the grasshopper diet breadth experiment. Juliane Heimann provided data on grasshopper phenology as well as data on grasshopper reproduction in 2007 for this manuscript. Jeanine Linz helped with the practical work in the field and in the lab. Günter Köhler and Carsten Renker provided the grasshopper abundance data and commented on earlier versions of this manuscript; Günter Köhler also supported the work in the lab. Claudia Stein provided data on vegetation height, plant species identity and abundance as well as LAI data; she also commented on the manuscript. Wolfgang W. Weisser was responsible for the implementation of the overall DIVA Jena research project. He contributed to the planning and execution of the experiment, gave advice on the statistics and commented an earlier version of the manuscript. **Manuscript status**: published in "Ecology" 2010

Chapter II: "Grasshopper fitness depends on parental experience"

Juliane Heimann, Sybille B. Unsicker & Wolfgang W. Weisser

In this study we conducted a relocation experiment where individuals of grasshopper populations from different meadows were transferred and caged on meadows along a gradient of plant species richness and composition. The goal of the present experiment was to discover of how well populations of the grasshopper species *C. parallelus* are established, expressed by fitness, in their meadow habitat and furthermore to reveal the importance of maternal effects due to parent feeding experiences.

I planned and performed the experiment, analyzed the data and wrote the manuscript. Sybille B. Unsicker and Wolfgang W. Weisser contributed to the planning and execution of the experiment, gave comments on the data analysis and gave advice on earlier versions of the manuscript. Furthermore Wolfgang W. Weisser was responsible for the implementation on the BIOLOG DIVA biodiversity project.

Manuscript status: in preparation for "Ecological Entomology"

Chapter III: "Rearing conditions affects grasshopper response to food plant diversity"

Juliane Heimann, Sybille B. Unsicker & Wolfgang W. Weisser

This laboratory feeding experiment was conducted to study the performance response of *laboratory-reared* and *field-caught* individuals of *C. parallelus* by feeding on a diverse *meadow diet* compared to a two-species *standard diet*. Furthermore to identify the importance of early larval (feeding) experiences for the response of *C. parallelus* grasshoppers originating from different populations feeding on a broad vs. a narrow diet was another goal of this study.

I planned and performed the experiment, analyzed the data and wrote the manuscript. Sybille B. Unsicker and Wolfgang W. Weisser contributed to the planning and execution of the experiment, gave comments on the data analysis and gave advice on earlier versions of the manuscript. Furthermore Wolfgang W. Weisser was responsible for the implementation on the BIOLOG DIVA biodiversity project. Furthermore Wolfgang W. Weisser was responsible for the implementation on the BIOLOG DIVA biodiversity project.

Manuscript status: in preparation for "European Journal of Entomology"

Chapter IV: "Taking Nutritional Ecology to the field: case studies with herbivorous insects"

Juliane Heimann, Spence T. Behmer, Wolfgang W.Weisser& Sybille B. Unsicker

At the end of the this thesis the presented experiments but also studies which has been done by others was integrated into a review article containing theoretical considerations about indirect effects of biotic and abiotic conditions on insect herbivore performance and the importance to combine knowledge of field and nutritional ecology.

According to my initial idea for this review article I was responsible for the literature research and wrote the manuscript. Sybille B. Unsicker and Wolfgang W. Weisser contributed to the planning of the article and gave comments on earlier versions of the manuscript. Spence T. Behmer revised an early version of this manuscript and gave furthermore comments on later versions of this article.

Manuscript status: in preparation for "Oecologia"

Glossary

experimental meadow:	six meadows, along a gradient of plant species richness, were *home* and *away* grasshopper populations have been caged on (see Chapter II)
meadow of origin:	meadows on which the parents have been caught (see Chapter II)
home grasshopper populations:	grasshopper populations which originated from the experimental meadow they have been caged on, these populations have never been relocated (see Chapter II)
away grasshopper populations:	grasshopper populations which do not originated from the experimental meadow on which they have been relocated on (see Chapter II)
meadow attributes:	certain attributes of grassland meadows that have any explanatory power for describing grasshopper performance like: plant species richness, plant species richness of grass, plant species richness of legumes, plant species richness of grass and legumes together, biomass, and also the fitness of the parental generation on each meadow (see Chapter II)
Delta attributes:	the difference in *meadow attributes* between *experimental meadow* and *meadow of origin* (see Chapter II)
laboratory-reared grasshoppers:	individuals hatched from eggs in the laboratory, laid by females caught on meadows in the study area one year before the experiment was started (see Chapter III)
field-caught grasshoppers:	third and fourth instar grasshoppers which have been caught in the study area by sweep netting in the year the experiment has been conducted (see Chapter III)
meadow diet:	contains the most abundant food plant species in the grasshoppers original habitat (see Chapter III)
standard diet:	consisting of only two food plant species (*Dactylis glomerata* and *Trifolium pratense*) (see Chapter III)
(grasshopper) *origin*	refers to the origin of the grasshoppers, either reared in the laboratory (*laboratory – reared*) or caught in the field (*field caught*) (see Chapter III)

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General Introduction

Insects in general – generalists in particular

Due to the great number of herbivore insect species living on and from their host plants the impact of food plant quality, diversity and composition on herbivore performance is a key interest in ecological research. In this context, the relationship between insects and plants is characterized by the approximated amount of 400.000 herbivorous insects which are living on more of 300.000 vascular plants (Schoonhoven et al., 1998) whereas, the most species of any organism class on planet earth belongs to insects, and the greatest part of land biomass is provided by green plants. In this regard the question after the effect of diversity on plant insect- relationships has been addressed by studies dealing with the interaction between plant diversity and the diversity of insect herbivores (e.g. Haddad et al., 2001; Koricheva et al., 2000; Mulder et al., 1999; Scherber et al., 2010; Siemann et al., 1998; Unsicker et al., 2006; Weisser & Siemann, 2004).

From the top-down point of view insect herbivores annually remove an average of 10% of plant biomass (Crawley, 1983; Scherber et al., 2010; Schoonhoven et al., 1998) which considerably varies according several biotic and abiotic factors (Scherber et al., 2010). The bottom up effects of plant resource quality on insect herbivores are among the three most important factors influencing insect herbivore abundance and performance in terrestrial ecosystems due to the water, nutrient and secondary compound content of food plant resources (Ritchie, 2000). On the top of this Price et al. (2002) states that plant-insect food webs represents more than 40% of global terrestrial biodiversity (Novotny & Basset, 2005). As many biologists discovered the herbivore plant system as an ideal model system to study fundamental aspects of ecology, evolution and ecological physiology (Schoonhoven et al., 1998) it is finally clear why so much interest has been appeared in working on insect-plant relationships.

By feeding on green plants herbivorous insects shows a degree of dietary specialization or generalization from monophagous species (feeding on a few closely related plant species) over oligophagous species (feeding on a number of plant species which all belong to the same plant family) to polyphagous species (feeding on many plant species belonging to different plant families)(Schoonhoven et al., 1998), although the majority of herbivores are not that easy to classify into this system. Most studies therefore rather distinguish specialist and generalist insect herbivores, classified after a very narrow vs. a more broad food plant spectrum. However, the degree of dietary specialization is important to disentangle biodiversity effects on insect community-levels, as the predicted response on plant species loss differs between specialized and generalized insect herbivores. For insect specialists the clearly predicted response is to decrease in abundance with

increasing plant diversity (Hector et al., 1999; Otway et al., 2005; Tahvanainen & Root, 1972). This assumption is based on Roots 'resource concentration hypothesis' which states that herbivores find and remain on plants more often in pure stands than in communities consisting of more plant species(Root, 1973). In contrast, no general theory concerning the influence of plant diversity on generalist insect herbivores exists. Generalist insect herbivores have been shown to cope with highly variable host plant quality (e.g. Bernays et al., 1994; Bernays & Chapman, 1970a; b; Berner et al., 2005; Miura & Ohsaki, 2004; Raubenheimer & Jones, 2006; Raubenheimer & Simpson, 2003; Simpson et al., 2004; Unsicker et al., 2008) a phenomenon often explained by the dietary mixing hypothesis.

Dietary mixing hypothesis – a predictor for generalist insect herbivore performance in habitats diverse in food plant species?

Broadening the diet by constantly switching between complementary food plants describes the theory of dietary mixing to balance nutrients and/or to dilute toxins in the food and to enhance or maintain fitness in generalist insect herbivores (Bernays et al., 1992; Kaufmann, 1965; Macfarlane & Thorsteinson, 1980; Pulliam, 1975; Rapport, 1980). 'Nutrient complementation' and 'toxin dilution' are the two hypotheses which have been brought forward to explain the beneficial effects of dietary mixing on generalist insect herbivores. To reach an optimal nutrient intake and therefore a good performance, insect herbivores expand their diet as it is assumed that a single food plant does not contain all the necessary nutrients ('nutrient complementation hypothesis') (Pulliam, 1975; Rapport, 1980). Selective feeding on the basis of nutrient regulation in unbalanced diets has been shown in studies with grasshoppers (Simpson & Raubenheimer, 1993), aphids (Abisgold et al., 1994) and caterpillars (Simmonds et al., 1992; Waldbauer et al., 1984). Plant toxins are diluted by mixing up single food plants whereas the negative effect caused by the toxin is supposed to decrease ('toxin dilution hypothesis') (Behmer et al., 2002; Freeland & Janzen, 1974; Marsh et al., 2006; Singer et al., 2002). To distinguish if the nutrient complementation or the toxin dilution hypothesis was decisive to achieve a fitness benefit due to a mixed diet is not trivial (Hagele & Rowell-Rahier, 1999). Still, the mechanism of dietary mixing has been shown in several studies with herbivorous insects, either by feeding them with real food plants (Bernays et al., 1992; Bernays et al., 1994; Hagele & Rowell-Rahier, 1999; Miura & Ohsaki, 2004; Unsicker et al., 2008) or on artificial diets(Behmer, 2009; Bernays et al., 1994; Chambers et al., 1995; Howard et al., 1994; Raubenheimer & Simpson, 2003; Waldbauer et al., 1984). In most feeding experiments the performance (growth rate, survival, fecundity) of generalist insect herbivores, mostly grasshoppers or lepidopteran, was enhanced on mixtures of diets (Bernays & Bright, 1993; Bernays et al., 1994; Hagele & Rowell-Rahier, 1999; Miura & Ohsaki, 2004; Unsicker et

al., 2008), but studies showing no benefit of dietary mixing can also be found in the literature (Bernays & Minkenberg, 1997; Singer, 2001).

Nevertheless, the mechanism of dietary mixing can serve as a predictor for generalist insect herbivore response on increased plant species richness, especially if nutrients are unevenly distributed among plants and habitats, both in space and time (Behmer et al., 2002; Gusewell & Koerselman, 2002; Joern & Behmer, 1998; Oleksyn et al., 2002; Osier & Lindroth, 2001; Raubenheimer & Simpson, 2003; von Fircks et al., 2001; Westoby, 1978). Feeding on a broader spectrum of food plants should generally increase the probability of obtaining a well- balanced mixture of macronutrients for generalist insect herbivores.

Within the discussion of dietary mixing and self-regulation of nutrients the hypothesis of nitrogen limitation in insect herbivores is often debated. The 'nitrogen limitation hypothesis' states that the presence and concentration of nitrogen in food plants is the most important factor influencing herbivore performance (Davison, 1995; Mattson, 1980; White, 1993) which was supported by several studies (Davison, 1995; Heidorn & Joern, 1987; Joern & Behmer, 1997; Ritchie, 2000). Although herbivores show indeed a higher nitrogen limitation by feeding on plants which contain far more carbohydrates than nitrogen, studies showed contrary results to the nitrogen limitation hypothesis and a uniform explanation is not likely for all herbivorous insects (Cease et al., 2012; Fischer & Fiedler, 2000; Joern & Behmer, 1998; Ritchie, 2000). Overall, the results rather emphasizes that nitrogen content alone is a weak predictor of food plant quality and that other plant nutrients, plant secondary metabolites and physical properties as well as abiotic conditions like temperature and solar radiation influences food plant quality and are at least as important for herbivore performance.

Constraints in the field - generalist insect herbivore performance under (semi -) natural conditions

Foraging under more natural conditions by feeding on real food plants comprises far more for herbivorous insects than only regulating nutrients to achieve a good performance. This fact is emphasizing the circumstance that experiments dealing with mechanism of nutritional balancing and dietary mixing in insect herbivores has been mostly conducted in the laboratory using artificial food or in some few studies also real food plants. Aspects like secondary compounds in the food plants, differences in food plant diversity and composition as well as direct and indirect effects of biotic and abiotic conditions (predation and anti-predation behavior, temperature, solar radiation, moisture, CO_2 elevation) are influencing insect herbivore performance in the field and were thereby broadly

neglected in laboratory experiments. Foraging in the field is therefore not only restricted by changed food plant quality due to abiotic factors but also inhibited by predation and anti-predation behavior which leads to the fact that the insect herbivore can not freely forage on every food plant existing in its habitat.

Generalist insect herbivores are able, due to a broader food plant spectrum, to exist in habitats that differ in food plant species diversity and composition. In grasslands for example – a typical habitat for a great number of generalist insect herbivores - plant species richness is a function of land use and abiotic conditions (Unsicker 2010). Apart from the direct effect of abiotic conditions on generalist insect herbivore performance, herbivores have to deal with changed food plant quality due to the effect of temperature, solar radiation and elevated CO_2 in different grassland habitats. In contrast to highly controlled laboratory studies it is important to keep in mind that by conducting experiments under natural (e.g. field observations) or even semi- natural conditions (e.g. cage experiments in the field, laboratory feeding studies with real food plants) all these factors might act on herbivore performance.

Study organism and Study area

The study organism used for the presented studies is the meadow grasshopper *Chorthippus parallelus* (Zetterstedt), which is one of the most abundant grasshopper species throughout Europe. This grasshopper occurs in different grassland habitats from wet meadows, moist and semi-dry grasslands, along waysides and gramineous forest tracks, in orchard meadows and sometimes on bog meadows (Ingrisch & Köhler, 1998). *Chorthippus parallelus* is known to feed preferentially on grasses but it has been observed that it´s diet also contains a number of forbs and legumes (Bernays& Chapman, 1970b; Franzke et al., 2010; Gangwere, 1961; Ingrisch & Köhler, 1998; Specht et al., 2008; Unsicker et al., 2008) thereby characterizing the species as a generalist insect herbivore. This grasshopper species has been served as study object for studies on dietary selection in insect herbivores (Bernays & Chapman, 1970a; b) and to study the use and consumption of food plants (Köhler, 1981). Furthermore, the fitness response of *Chorthippus parallelus* on food plant species richness from a broad vs. a narrow diet in the laboratory (Unsicker et al., 2008)and in a field biodiversity experiment (Specht et al., 2008) has been tested together with its feeding on varying food plant mixtures from different meadow habitats (Franzke et al., 2010). *C. parallelus* is very territorial and has, like most European grasshopper species, a low dispersal rate due to winglessness (Ingrisch & Köhler, 1998). The studies of this thesis have been conducted within the BIOLOG project (BIOLOgical diversity and Global change), a transdisciplinary scientific program which was funded from 2000 – 2010 by the Federal Ministry of

Education and Research. BIOLOG DIVA Jena, a long-term biodiversity experiment, as a part of the BIOLOG Europe project, tried to disentangle the relationship between biodiversity and ecosystem functioning in semi-natural grassland ecosystems. The study area which contained 20 hay meadows varying in plant species richness and composition was located in the Franconian Forest in Central Germany which is a low mountain range at the border of Bavaria and Thuringia (50°21'N and 11°00-11°37'E) (see **Figure 1**).A main selection criterion for these meadows was extensive management for at least the last decade, including no fertilization, no grazing, a moderate mowing schedule of two cuts per year and a pH value of above 5.0 (Kahmen et al., 2005).
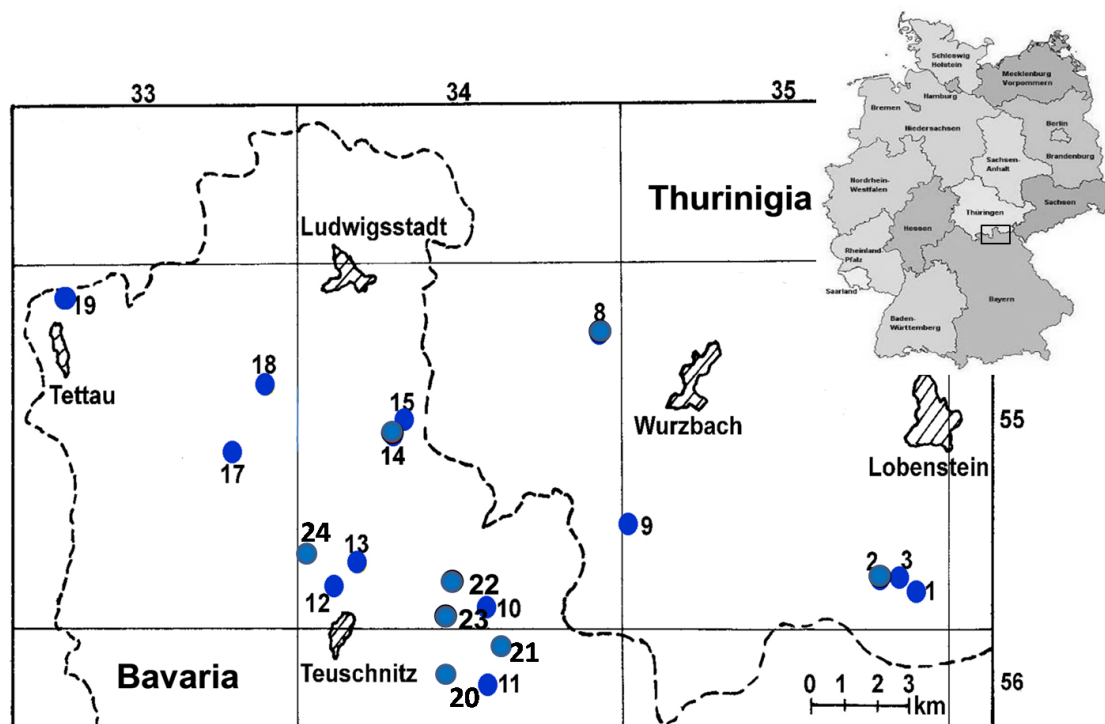

Figure 1: Extensively managed meadows (blue dots) in the study area of the Schiefergebirge/Franconian forest which is located at the border between Thuringia and Bavaria (black box in the general map of Germany).

Aim and Main Questions

Based on the studies of dietary mixing with generalist insect herbivores it can be assumed that feeding in a more diverse habitat with the possibility of finding more potential food plants increases generalist insect herbivores performance due to obtaining a well – balanced intake of macronutrients. Tests in field experiments (Pfisterer et al., 2003; Specht et al., 2008) and feeding experiments with natural or original food plants (from meadow habitats in the study area) under laboratory conditions (Unsicker et

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al., 2008; Franzke et al., 2010) shows contradicting results. Whereas the performance of the grasshopper species *Parapleurus alliaceus* increased with increasing plant species richness in a field biodiversity experiment (Pfisterer et al.) no such effect was shown in the grasshopper species *Chorthippus parallelus*(Specht et al., 2008). However, a clear indication for the positive effect of dietary mixing for generalist insect herbivore performance was given by the laboratory feeding experiment with *C. parallelus* by Unsicker et al. (2008). In contrast, no effects of composition and species richness in the diet on grasshopper performance were detected during another feeding experiment with *C. parallelus* (Franzke et al., 2010).

In the field experiment conducted by Specht et al. (2008) fourth instar nymphs of field caught individuals of *C. parallelus* had been transferred into cages to test the effect of an experimental gradient of plant diversity. However, neither plant species richness nor plant functional group richness affected grasshopper fitness (Specht et al., 2008) but it was indicated that, at least in the field, grasshopper fitness was more influenced by plant functional group identity, i.e. the presence of grasses, than by plant species richness *per se*. By feeding individuals of *C. parallelus* on diet mixtures consisting of one, three and up to an maximum of eight food plants throughout a grasshopper lifetime it was shown that grasshopper survival and fecundity was highest in the most diverse host plant mixture consisting of eight plant species offered and lowest in treatments with only single host plants (Unsicker et al., 2008). Furthermore a strong dependency of food plant choice on grasshopper developmental stage was revealed. In the study by Franzke et al. (2010) individuals of *C. parallelus* were fed on food plant mixtures containing 11 to 15 food plant species simulating four different grassland habitats from the BIOLOG study area. Although no difference in grasshopper performance occurs by feeding on the four different diets the study revealed differences in the conversion efficiency to body mass explained by selective dietary mixing which supports the active encounter of quality deficiencies in the food. One possible explanation for the different results between the laboratory feeding studies of Unsicker et al. (2008) and Franzke et al. (2010) could be the total number of food plants in the diets. Franzke et al. (2010) tried to simulate natural conditions by offering the highest amount of food plants per meadow diet, from minimal 11 to maximum 15 plants per diet. In Unsicker et al. (2008) only mixtures from one, three to maximum eight food plants were offered throughout grasshopper lifetime and thus it is conceivable that beneficial effects through dietary mixing in grasshoppers are only evident below a certain threshold number of available food plants throughout grasshopper ontogeny. There are, however, a number of additional differences between these three studies conducted with *C. parallelus*. Differences might be derived by contrasting treatments of the study organism in the first nymphal stages. Grasshoppers were caught in the field before subjected to the experimental treatments in the study by Specht et al. (2008) whereas in Unsicker et al. (2008) effects of plant species richness were found in individuals reared from eggs in

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the laboratory. The early experience of nymphs in the field may therefore have affected subsequent results. As in fact literature showed very different performance response of the generalist insect herbivore *C. parallelus* on plant species richness and composition the aim of the present thesis was to further disentangle this relationship with field studies and laboratory experiments. Gaining knowledge about the mechanisms underlying generalist insect herbivore performance in natural systems was another goal of this thesis. Furthermore insect feeding ontogeny and early feeding experiences are often neglected while describing insect herbivore responses to food plant quality in the literature, although especially early instar feeding experiences have shown to be important for later insect development and fitness (Barrett et al., 2009; Colasurdo et al., 2009; Metcalfe & Monaghan, 2001; Unsicker et al., 2008). In this context, it was aimed to measure performance response of *C. parallelus* over several developmental stages within the conducted experiments of this thesis. To achieve these aims the scope of generalist insect herbivore performance response on food plant quality and diversity was expanded and several different topics like the influence of a broad vs. a narrow diet or the difference between field vs. laboratory conditions as well as the influence of early feeding experiences and maternal effects on generalist insect herbivore performance were highlighted. In this context an observational field study analyzed the fecundity of *C. parallelus* between 15 different meadow habitats, and a relocation experiment along a gradient of plant species richness tried to discover of how well (meta-) populations of this grasshopper species are established in their meadow habitats. A laboratory feeding experiment conducted with different grasshopper populations tried to figure out how *C. parallelus* fitness of *laboratory-reared* and *field-caught* individuals is varying by feeding on a diverse *meadow diet* compared to a two-species *standard diet*.

Under field conditions herbivore performance is often indirectly influenced by biotic and abiotic factors mediated through changed food plant quality (also see: *Constraints in the field- generalist insect herbivore performance under (semi-)natural conditions*). Due to the fact that the experimental part of this thesis containing studies conducted either in the laboratory but with real food plants or as cage experiment or as observational study in the field, the constraints herbivores are subjected to under these conditions have been especially noted and discussed in the framework of a theoretical review article. Furthermore this review article tries to expose knowledge gaps concerning the nutritional ecology of insect-plant interactions in the field and gives impulses to a new discussion by the suggestion to merge findings from two different ecological research fields.

The main questions which were asked in the thesis were:

(1) How does plant species diversity affect the performance of *C. parallelus* under field conditions?

(2) How well are different populations of *C. parallelus* established, expressed by fitness, in their meadows in dependence of plant species richness & composition and how important are maternal effects due to parental feeding experiences?

(3) How important are early larval (feeding) experiences for the response of *C. parallelus* grasshoppers originating from different populations feeding on broad vs. narrow diets?

(4) What is necessary to further disentangle generalist insect herbivore performance in the field by focusing on a strong influence of biotic and abiotic conditions on food plant diversity, quality and composition?

Chapter I

Plant species richness in montane grasslands affects the fitness of a generalist grasshopper species

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Abstract

Theory predicts negative effects of increasing plant diversity on the abundance of specialist insect herbivores, but little is known about how plant diversity affects the performance and abundance of generalist insect herbivores. We studied oviposition rates and offspring numbers in females of the generalist grasshopper *Chorthippus parallelus* that were collected in 15 montane grasslands in 2005 and 2007 along a gradient of plant species richness in Central Germany. In addition to plant species richness, we determined evenness and plant community composition in the grasslands, and measured above-ground plant biomass and other habitat variables such as leaf-area index, vegetation height, and solar radiation. There was substantial variation among sites in grasshopper fecundity and the number of nymphs that hatched from the egg pods. Both fitness measures were positively influenced by plant species richness at the sites, while female fitness did not correlate with any of the other habitat parameters. Abundance of *C. parallelus* in the grasslands was positively correlated with plant species richness, plant community composition, and incident solar radiation of the sites. There were no phenological differences between grasshoppers from the different study sites. Our results suggest that decreasing biodiversity threatens the persistence not only of specialist but also of generalist insect herbivores via a variety of mechanisms including a decrease in diversity of the generalists` food plants.

Keywords: extensively managed montane grasslands; fecundity; plant community composition; reproduction.

Introduction

Recently, increasing attention has been paid to the community-level effects of biodiversity (Hooper et al., 2005; Loreau, 2000). While earlier studies on the relationship between biodiversity and ecosystem functioning mostly focused on primary producer (e.g. Schulze & Mooney, 1993 and references therein), more recent studies have started to address the effects of species diversity on trophic interactions (e.g. Cardinale et al., 2006; Duffy, 2002 and references therein; Schmitz, 2003); for example, between plants and herbivorous insects (van Ruijven et al., 2005; Weisser & Siemann, 2004). With respect to herbivorous insects, research has focused on the effects of plant diversity on herbivore diversity or abundance (e.g. Andow, 1991; Jactel et al., 2005; Siemann, 1998). These studies were motivated by the work of Root (1973), who predicted that the abundance of herbivores will be lower in diverse plant communities than it is in simple communities ('resource-concentration-hypothesis'). The mechanism underlying this assumption is that herbivores find and remain on plants more often in pure stands than in diverse plant communities (Root 1973). Tests of Root's hypothesis have thus focused on the response of specialist insect herbivores to changes in plant diversity (e.g. Otway et al., 2005; Schellhorn & Sork, 1997), often in agricultural systems (Andow, 1991; Tonhasca & Byrne, 1994). However, it is essential to also understand the effects of plant diversity on generalist herbivore species, as they are an important part of natural ecosystems.

In contrast to the many theories about specialists, there is no general theory of how increasing plant diversity might affect the abundance of generalist herbivores. Predictions can be derived from ecophysiological studies investigating the effects of mixed diet on the performance of generalist insect herbivores (e.g. Bernays & Chapmann, 1994; Simpson & Raubenheimer, 2000). In most of these laboratory studies, the performance (e.g. survival, growth rate, fecundity, etc.) of generalist herbivores was better on mixtures of food plant species than on diets containing single plant species (e.g. Bernays & Bright, 1993; Unsicker et al., 2008), although there were exceptions in which no general benefits of dietary mixing were measurable (e.g. Bernays & Minkenberg, 1997; Singer, 2001).

Based on our results from these ecophysiological studies with generalist herbivores in the laboratory, we can predict that under natural conditions in the field, generalist herbivores perform better in more diverse habitats where they can take advantage of many species of potential food plants. Experimental tests of this prediction in biodiversity experiments involving grasshoppers reared outdoors on mixtures of different diets have led to conflicting results (Pfisterer et al., 2003; Specht et al., 2008). Plant diversity in natural habitats is often a function of land use and abiotic conditions, all of which are likely to affect herbivore performance. To separate the effects on herbivore fitness of land use and climate (e.g. vegetation structure and solar radiation) from the effects on herbivore fitness of

diet (e.g. diversity and abundance of food plant species), it is essential to make simultaneous measurements of variation in all these parameters.

In Central European grasslands, grasshoppers (Orthoptera, Acrididae) are an important component of the phytophagous insect community and are responsible for a considerable part of total herbivory (Köhler et al., 1987). One of the most common acridid species in Central Europe is the meadow grasshopper *Chorthippus parallelus* (Acrididae, Gomphocerinae; Zetterstedt 1821) which has been the subject of several classical studies on diet selection in herbivorous insects (Bernays & Chapman, 1970a; b) as well as of studies relating to the consumption and use of food (Köhler & Schäller, 1981).

We tested the influence of plant species richness on the fitness and the abundance of *C. parallelus* by collecting females in meadows that were selected along a gradient of plant species richness (Kahmen et al., 2005). In addition to plant species richness, we recorded plant community composition, plant community biomass, vegetation height, leaf area index, and solar radiation; these are parameters that can affect grasshopper performance. The main questions we asked were

(1) Is there variation in fitness measures such as fecundity and number of hatched offspring of female *C. parallelus* among the different grassland sites?

(2) Does grasshopper fitness correlate with plant species richness?

(3) What are the effects of other habitat variables for grasshopper performance, compared to the influence of plant species richness?

Materials and methods

Study sites

The study was conducted in 15 hay meadows in Frankenwald and Thüringer Schiefergebirge (11°00'-11°37'E and 50°21'-50°34'N), a low mountain range at the Bavarian / Thuringian border in Central Germany with a maximum elevation of 841 m above sea level. The 15 grassland sites are part an interdisciplinary research project. Selection criteria for the sites were (1) no fertilization and (2) no grazing during the last 15 years and (3) soil pH higher than 5.0 (Kahmen et al., 2005a). All of the meadows have been extensively managed for at least the last 15 years with two mowings per year (June/July and August/September). Sites were chosen in order to represent a gradient of plant species

richness from about 20 species (species in four plots of one square meter each) in the species poor meadows to about 41 species in the most diverse mountain hay meadows.

Habitat variables

All habitat variables were measured in June 2005 in a 5m^2 plot in each of 15 field sites. Aboveground community biomass was sampled in four 0.1m^2 rectangles (total 0.4 m² for each plot) quadrats within the 5x5 m plot, by cutting the vegetation 3 cm aboveground. The community biomass samples were dried at 70°C for 48 h and weighed. All plant species were identified in four 1x1 m quadrats that were placed close to the center of the 5x5 m plot and percent cover was visually estimated using a modified Londo scale (Londo 1976). We use plant species richness as the average species richness in these four quadrats.

The leaf area index (LAI) of each study site was measured four times in a 5 x 5 m plot with a LAI-2000 Plant Canopy Analyzer (LI-COR Biosciences, USA) and the maximum height of the plant community at each site was determined by placing a quadratic piece of polystyrene (30 x 30 cm) on top of the vegetation. The height at which the quadrat rested on top of the vegetation was measured. This measure was repeated at four points with a distance of 1.5 m from each other, and averaged. To characterize the sites, geographic position (GPS coordinates), altitude (range: 595-685 m above sea level), exposition (range: pure North to pure South) and inclination (range: 0-20°) were determined. Based on exposition and inclination, we calculated mean potential direct solar insolation (PDSI; Homann, Schumacher and Perner, unpublished software program, algorithm based on Volz 1959). In addition to species richness, we calculated Carmargo's evenness as another relevant plant diversity measure (Krebs, 1999).

Grasshopper abundances in the study area

At June 17 and 18 in 2005, abundances of *C. parallelus* in 11 study sites were classified based on the sampling success of two people performing sweep netting for 30 min within a 300 m² portion of each meadow. Eight categories were used to measure grasshopper abundances: 0: no record; 1: 1-4 individuals; 2: 5-10; 3: 11-20; 4: 21-30; 5: 31-40; 6: 41-50; 7: 51-60; 8: 61-70 individuals. For all further statistical analyses, the highest number of each category was picked (10, 20, 30, 40, 50, 60 and 70).

Experimental setup and grasshopper size

In order to determine fitness parameters in *C. parallelus* along the gradient of plant species diversity, we collected 20 adult females of *C. parallelus* in each of the 15 meadows (= 300 females) at the end of July 2005 (July 26 and 28). Females of one site were placed in plastic boxes (27x17x18 cm Savic, Belgium), together with 5-10 males that were caught additionally at each site. Mating of grasshoppers caught in the same site was allowed to take place until the start of the experiment. Twenty-four hours after the grasshoppers were caught, each female was placed individually in a cage, 14 cm in diameter and 17 cm in height, consisting of aluminum mesh (aperture size: 1.5 mm) fixed to the lid of a Petri dish (14.5 cm in diameter) with hot-melt adhesive, thus forming a cylinder that slides over the bottom part of the Petri dish. Each cage contained a little plastic cup (diameter: 5 cm, height: 3 cm) filled with a 50/50 mixture of moistened sand and soil for egg-laying, and a small plastic vial for holding the food plant.

Throughout the experiment, all grasshoppers were fed with *Dactylis glomerata* (Poaceae), a grass species known to be an adequate food source for *C. parallelus* (Ingrisch & Köhler, 1998; Köhler, 2001; Unsicker et al., 2008). The freshly cut grass leaves were put in a plastic vial that was filled with water to prevent the grass from wilting. In order to provide sufficient amount of food throughout the experiment, the cages were checked daily and if necessary, freshly cut *Dactylis glomerata* leaves were added. The cups with the sand-soil mixture were also kept moist throughout the experiment. As not all females could be handled within one day, 144 females (= 50.2%) were kept individually in cages for six days and 143 females (= 49.8%) for seven days. Thereafter females were anaesthetised with CO_2 and the length of the left hind femur was measured under a binocular microscope with the help of an object slide graved with measuring units. As landmark points for the measurement, we chose the most distant points of the outer rim of the upper, larger lobe of the notched base and the outer rim of the upper one of the two genicular lobes at the apex (Jentzsch et al., 2003).

Grasshopper reproduction

In 2005, grasshopper fecundity (egg pod number) and the number of hatchlings from the egg pods were taken as fitness measures. Egg pods laid by each female until the end of the experiment (after 6-7 days) were counted by carefully washing the egg pods out the egg-laying soil. After counting, the egg pods were buried again in an autoclaved sand/soil mixture (50/50) keeping the same orientation as they had before with the narrow part of the egg pod facing upwards. The pods laid by a single female were placed together in one cup covered with conventional fly mesh to prevent grasshopper progeny

from escaping after hatching. For two months the egg pods were kept in the lab at room temperature so that the embryos could develop to blastokinesis. Throughout this time, the sand/soil mixture in the cups was regularly watered to prevent drying out. At the beginning of October 2005 all cups with egg pods were transferred to the refrigerator at 6°C. On January 26 all cups were removed from the refrigerator and kept under lab conditions with an average diurnal room temperature of 22°C ± 1.6°C (mean ± SD). All pots containing egg pods were checked daily for hatchlings and were watered when necessary. Freshly hatched grasshoppers were collected with an aspirator and immediately killed by freezing at -20°C. As the experiment was not terminated at the same day for all female grasshoppers, the rates per day for both pods laid and hatched offspring were calculated. Thus for each female, egg pods per day and number of offspring per day (= number of hatchlings divided by time for oviposition) were calculated as variables measuring fecundity and reproductive success respectively.

In 2007, we repeated our study with slight modifications in order to test if the relationship for grasshopper fecundity and plant species richness is reproducible. In mid- July 2007 (July 13 and 17) 10 to 30 juvenile grasshoppers (nymphal stage 3 and 4) were caught in 9 of the 15 study sites from 2005. The investigation of grasshopper abundance in 2007 was part of another research project, which is the reason, why the number of field sites investigated differed from the 2005. The nymphs that were caught in each site were kept in separate cages (30x30x30cm, MegaView Science Co., Ltd., Taiwan) under laboratory conditions. In 2007 the grasshoppers were fed with a food plant mixture consisting of the grass species *D. glomerata* and the legume *Trifolium pratense.* After maturity moult (end of July until middle of August) grasshopper males and females that were originally caught in the same study site were allowed to mate and oviposit in cups filled with a sand/soil mixture, just as it was described above. The oviposition experiment in 2007 lasted until the grasshoppers died. Thereafter the number of egg pods laid by the females in each cage was counted. By dividing the total number of egg pods through the number of female grasshoppers present in the cages (cages represent study sites), the number of egg pods per female grasshopper was calculated. In 2005 twelve female grasshoppers died during the experiment and one escaped, hence the number of valid replicates for the statistical analysis was 287.

Grasshopper phenology

Because in 2005 grasshoppers were only counted without determining the nymphal stages of each individual, another survey was conducted in 2007 in order to test for differences in grasshopper phenology between sites. We caught *C. parallelus* grasshoppers by sweep netting along a transect in

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each of the study sites at the beginning of July 2007 (July 7). Ten beats with the sweep net were performed in each site, and the developmental status of each *C. parallelus* grasshopper caught was then recorded (all instars but the first could be found in the study sites).Thus the phenological status of each grasshopper population could be determined.

Grasshopper diet breadth experiment

To test the diet breadth of *C. parallelus* and to verify the fact that this grasshopper species is a generalist feeder rather than a specialist, we performed a cage experiment in which six grasshopper females were continuously provided with food plants of a species rich meadow typical for the study area. Thirteen plant species were selected as food plants based on their abundance in the study site. All plant species in the grassland that had a cover over 1% (plant cover was estimated in four 1m² plots) were integrated in the feeding experiment. The selected plant species were seven grass species (*Agrostis tenuis*, *Anthoxanthum odoratum*, *Dactylis glomerata*, *Festuca rubra*, *Holcus lanatus*, *Phleum pratense* and *Trisetum flavescens*), four herbaceous species (*Alchemilla vulgaris*, *Hypericum maculatum, Plantago lanceolata* and *Veronica chamaedrys*) and two legumes (*Trifolium pratense* and *Trifolium repens*).

Grasshoppers were reared from oothecae laid in the laboratory by females collected from a field population near Jena in 2004. Individuals were kept singly in 6L Fauna boxes (Savic, Belgium) from within three days after hatching until death. Freshly cut food plants were randomly arranged in a plastic box (115 x 15 x 60 mm) placed in the Fauna box, filled with water and covered with plastic foil to prevent plants from desiccation and grasshoppers from drowning. Plants were offered *ad libitum*.

Every fourth day, the leaf area (in mm²) of each plant species consumed by the grasshoppers was measured with the help of graph paper. To convert the leaf area eaten into dry weight consumed ten 1cm² leaf discs of each plant species were cut, dried for 48h in a drying oven at 70°C and weighed. Here, we only present data on food plant consumption of females during their four larval instars.

Statistical analysis

All count data were square-root transformed prior to analyses. Means are always displayed with standard errors (SE).

To account for the effects of plant community composition on the fitness of *C. parallelus*, we used non-metric multidimensional scaling (NMDS) ordination of the plant communities in the 15 study sites, using the square-root-transformed plant cover data. NMDS is an ordination method that has been has been shown to deliver robust results for vegetation data (Michin 1987, Faith et al. 1987). From a matrix of resemblances (similarities or dissimilarities) between pairs of objects, here plant communities, NMDS constructs a configuration of points in a specified number of dimensions, such that the rank order agreement between the inter-point distances and the resemblance values is maximized. As a distance measure, the Bray-Curtis coefficient was used and NMDS analyses were conducted using the program PC-ORD (McCune & Mefford, 1997). We tested if a two-dimensional solution does give a sufficiently good fit to the data, indicated by a low 'stress'-value (Faith et al., 1987).

Because we investigated eight habitat variables (plant species richness, community biomass, Camargos's evenness, NMDS1, NMDS2, LAI, vegetation height and solar radiation; **Table 1**) we tested for correlations among the independent variables using a principal component analysis (PCA).

Multiple linear regressions in a stepwise selection procedure were carried out using SPSS 15.0 for Windows (SPSS Inc.) to analyse the effects of habitat variables on grasshopper fitness parameters and abundances. In addition to the models presented by the stepwise procedure, models were tested by using the forward and backward procedure implemented in SPSS. The AIC (Aikaike's Information Criterion) values of the best model are reported.

Results

Grasshopper diet breadth experiment

Female grasshoppers in the experiment lived on average for 97.5 ± 0.89 days. **Figure 1** shows the larval food consumption of six female grasshoppers from the first nymphal instar to the time of maturity moult (45.0 ± 0.97 mg). During their development, the grasshoppers consumed all offered plant species except the herbaceous species *Alchemilla vulgaris* (**Figure 1**). There were significant differences in the amount of food consumed from individual plant species (ANOVA: $F_{12,\ 5}$=25.4, *p*=0.001). The bulk of biomass consumed during the four larval stages was grasses (87.4%) and, to a lower extent, herbs (12.6%). The two legume species *Trifolium pratense* and *Trifolium repens* comprised 12.5% of the total nymphal food consumed. Due to these results, *C. parallelus* can be termed a true generalist feeding on food plants from more than one plant family.
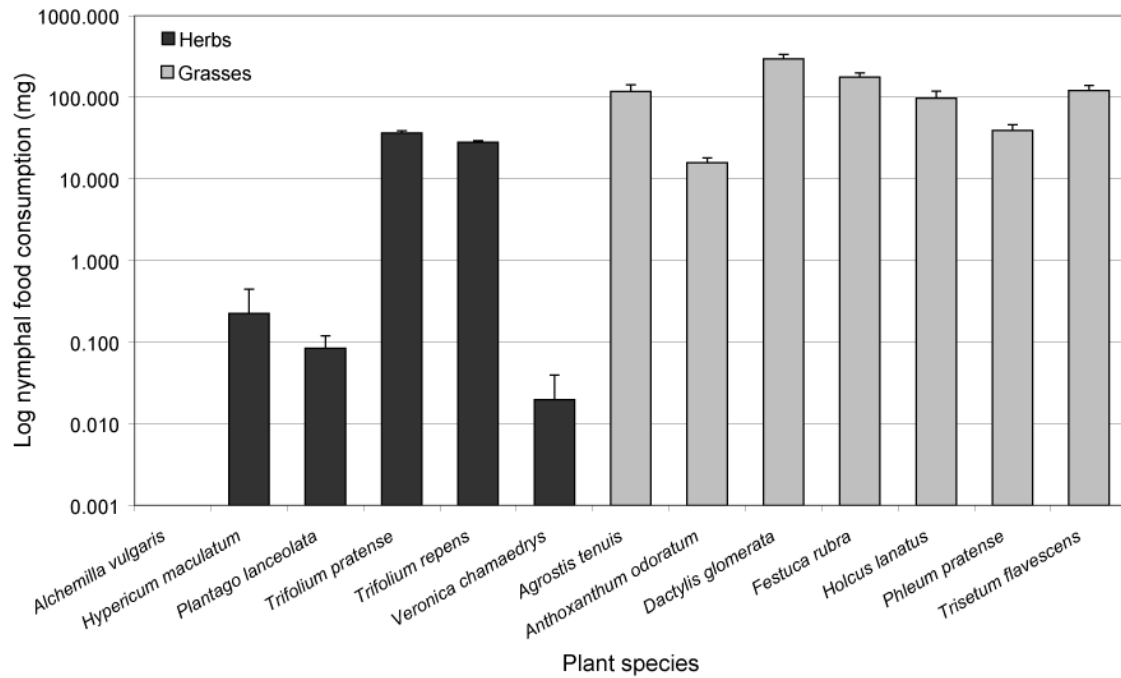
Figure 1: Food consumption of six females (mean ± SE) of *C. parallelus* during the four nymphal instars in a food plant mixture with seven grasses and six herbs. Note that the y-axis is in log scale.

Multivariate analysis of plant communities

In total 89 plant species occurred in the 15 study sites and their abundances were used for the ordination of the plant communities. The NMDS analysis showed that a two-dimensional solution was sufficient to achieve low stress values explaining plant species composition in the 15 sites (first axis/dimension = 34.7, second axis/dimension = 9.0). Thus for all future analyses, we used the two main axes, labeled NMDS1 and NMDS2, to represent plant community composition. For a more detailed description of the occurrence of particular plant species along the NMDS gradients please see Kahmen et al. (2005). The relationships between the plant species that were tested in the grasshopper diet breadth experiment and the two NMDS axes are shown in the supplementary material (**Table S1**).

Multivariate analysis of habitat variables

The means, standard errors and ranges of all measured habitat variables are given in **Table 1**. Community biomass decreased with increasing plant species richness, with 652.04 g m^{-1} and 120.74 g m^{-1} at the site with the lowest (20 species) and highest diversity (41 species), respectively. However, this relationship was not significant ($r^2 = 0.26$, $p = 0.062$). There was no relationship between plant species richness and Camargo's evenness ($r^2 < 0.01$, $p = 0.736$). The number of grass species was positively correlated with the total number of plant species ($r^2 = 0.734$, $p < 0.001$). Community biomass was correlated with leaf area index (LAI), vegetation height and NMDS1, whereas NMDS1 was correlated with Camargo's evenness.

We included the two NMDS axes NMDS1 and NMDS2 into a PCA analysis of all habitat variables. The resulting first two PCA axes explained 70% of the total variance in habitat variables (**Table 2**). The first axis was closely positively correlated with community biomass, LAI and vegetation height, and negatively correlated with NMDS2. Thus, the composition of the plant community, expressed by NMDS2, affected productivity and structural parameters of the plant community (biomass, LAI, height). The second axis was mainly determined by NMDS1 and Camargo's evenness.

To reduce the number of parameters in further multiple regressions, Community biomass was selected instead of the closely correlated vegetation height and LAI. While NMDS2 was correlated with community biomass (**Table 2**), both NMDS1 and NMDS2 were kept for future analyses as plant species composition represents the host plant combinations in the plant communities, i.e. resource quality, whereas productivity is a measure of resource quantity. The six parameters plant species richness, Camargo's evenness, community biomass, NMDS1, NMDS2 and solar radiation were used as independent variables in all multiple regressions on grasshopper abundance and fitness-related variables.

Table 1: Summary of data for eight habitat variables that were measured in the 15 study sites in June 2005.

Parameter	Mean ± SE	Range	n*
Plant species richness	30.4 ± 1.58	20 - 41	15
Community biomass (g m^{-2})	298.49 ± 36.53	120.74 – 652.04	14
NMDS1	0.02 ± 0.12	-0.85 – 0.96	15
NMDS2	-0.03 ± 0.24	-1.68 – 1.06	15
Camargo´s evenness	0.32 ± 0.015	0.23 – 0.45	15
Leaf area index (LAI)	3.68 ± 0.37	1.18 – 5.92	14
Vegetation height (cm)	30.57 ± 6.7	16.0 – 108.0	14
Solar radiation in site (J / cm²)	2055.68 ± 32.1	1796.97 –2353.15	15

* the differences in the number of replicates for the habitat variables is due to early accidental mowing of one study site in June 2005. Thus none of the parameters but plant species richness and solar radiation could be investigated at this time.

Grasshopper abundances

There was a positive relationship between plant species richness and grasshopper abundance in June 2005 (r^2 = 0.53, $F_{1,9}$ = 10.12, p = 0.011, **Figure 2**). Increasing community biomass tended to decrease the abundance of grasshoppers but the relationship was marginally significant (r = 0.40, $F_{1,8}$ = 5.29, p = 0.05). There was no relationship between grasshopper abundance and Camargo's evenness (r^2 = 0.02, $F_{1,9}$ = 0.2, p = 0.663) or NMDS1 (r^2 = 0.09, $F_{1,9}$ = 0.86, p = 0.377), but *C. parallelus* abundances were influenced by NMDS2 (r^2 = 0.55, $F_{1,9}$ = 11.05, p = 0.009) and solar radiation (r^2 = 0.42, $F_{1,9}$ = 6.46, p = 0.032) in the sites. There was a positive relationship between grasshopper abundance and solar radiation. In a multiple linear regression with the six selected habitat variables, only plant species richness and NMDS2 remained in the final model (species richness alone: r^2 = 0.70, $F_{1,8}$ = 18.74, AIC = 51.04, p = 0.003; plant species richness and NMDS2: r^2 = 0.85, $F_{2,7}$ = 20.44, AIC = 45.88, p = 0.001).

Table 2: Eigenvalues and eigenvector coefficients (loadings) of a standardized principal component

analysis (PCA) of the eight independent variables. Loadings > 0.5 are bold to emphasize the impact of the representative axes.

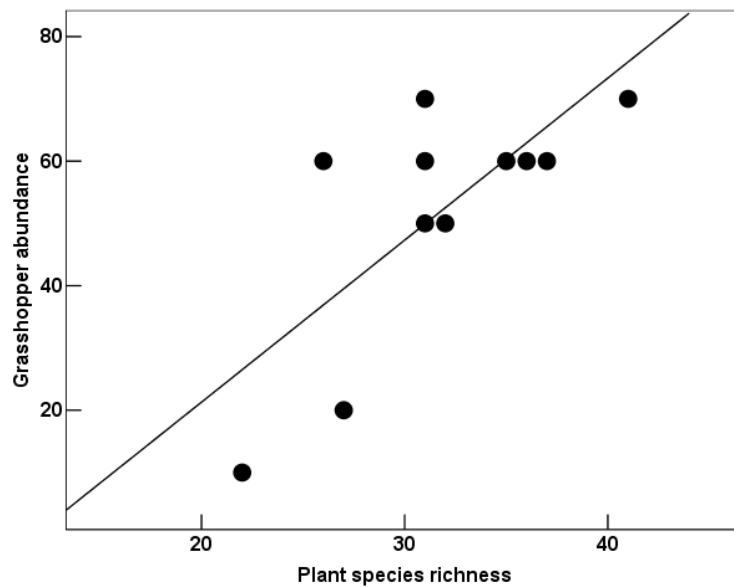|	PCA1	PCA2
Eigenvalues	*0.5062*	*0.1904*
Plant species richness	**-0.573**	-0.161
Community biomass	**0.943**	0.089
Camargo´s evenness	0.002	**-0.865**
NMDS1	0.126	**0.800**
NMDS2	**-0.914**	0.046
Leaf area index (LAI)	**0.893**	-0.150
Solar radiation	**-0.592**	0.271
Vegetation height	**0.914**	0.066

Figure 2: Relationship between plant species richness and the abundance of *C. parallelus* in 11 study sites. R^2 = 0.53, $F_{1,9}$ = 10.12, p = 0.011. Abundance is the number of grasshoppers caught in 30min by two people sweep-netting along two transects in the respective study site.

Grasshopper body size

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Mean female femur length was independent of overall plant species richness ($r^2 < 0.001$, $F_{1,13} < 0.001$, $p = 0.988$), community biomass ($r^2 = 0.03$, $F_{1,12} = 0.32$, $p = 0.581$), NMDS1 ($r^2 = 0.001$, $F_{1,13} = 0.01$, $p = 0.911$), NMDS2 ($r^2 = 0.01$, $F_{1,13} = 0.2$, $p = 0.658$) and solar radiation ($r^2 = 0.004$, $F_{1,13} = 0.05$, $p = 0.833$), but there was a significant positive relationship between Camargo's evenness and mean femur length in females of *C. parallelus* ($r^2 = 0.55$, $F_{1,12} = 16.12$, $p = 0.001$). In a multiple regression the best model included Camargo's evenness and NMDS1 as explanatory variables (Camargo's evenness alone: $r^2 = 0.55$, $F_{1,12} = 14.74$, AIC = -43.76, $p = 0.002$; Camargo's evenness and NMDS1: $r^2 = 0.71$, $F_{2,11} = 13.96$, AIC = -48.23, $p = 0.001$).

Grasshopper reproduction 2005

Females laid between zero and two egg pods within the timeframe of seven days, and 52.3% of the 287 grasshoppers laid at least one egg pod (193 eggs pods by n = 150 females). The percentage of females laying zero egg pods was negatively correlated with plant species richness of the study sites ($r^2 = 0.33$, $p = 0.025$). There was a significant positive relationship between mean egg pod number per day and overall plant species richness across sites ($r^2 = 0.39$, $F_{1,13} = 8.39$, $p = 0.012$, **Figure 3a**). In a multiple regression with the six habitat variables only plant species richness emerged as significant predictor variable ($r^2 = 0.426$, $F_{1,12} = 8.891$, AIC= -85.76, $p = 0.011$).

Hatching started 18 days after the egg pods were removed from the refrigerator. Offspring hatched from 134 of the 193 egg pods laid by 99 of the 150 females. Altogether 716 larvae hatched from the 134 egg pods (3.68 ± 0.28 individuals per egg pod). Grasshopper abundances of *C. parallelus* in the field did not influence the number of offspring per day ($r^2 = 0,113$, $p = 0.312$, $n = 11$). There was a significant positive relationship between plant species richness and the mean number of offspring per day ($r^2 = 0.30$, $F_{1,13} = 5.52$, $p = 0.035$, **Figure 3b**). In a multiple regression with the six selected habitat variables, only plant species richness was significant ($r^2 = 0.30$, $F_{1,12} = 5.10$, AIC = -61.42, $p = 0.043$).

Grasshopper reproduction 2007

There was a significant positive relationship between mean egg pod numbers per female grasshopper and overall plant species richness in their original habitats ($r^2 = 0.56$, $F_{1,7} = 7.624$, $p = 0.028$). In a multiple regression with the six habitat variables only plant species richness was retained as a significant predictor variable ($r^2 = 0.56$, $F_{1,6} = 6.535$, AIC= 2.876, $p = 0.043$).
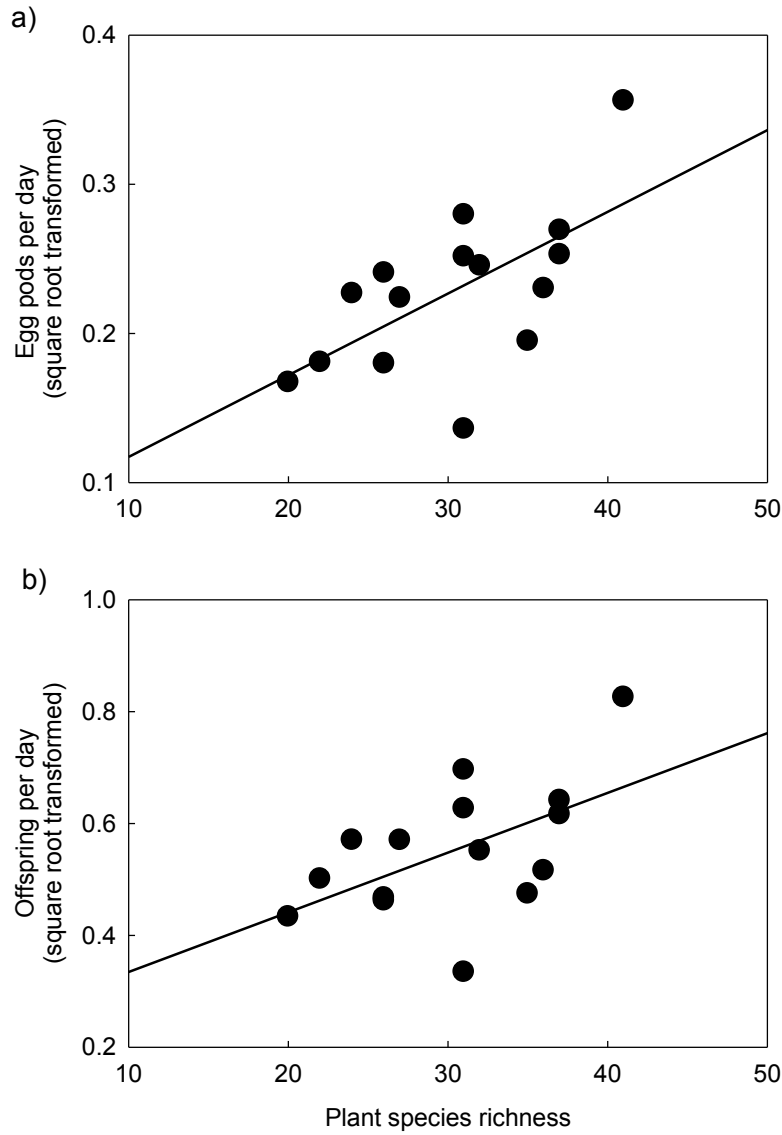
Figure 3: Relationship between plant species richness and **a)** female fecundity (mean pod number per day) in 15 study sites. $R^2 = 0.39$, $F_{1,13} = 8.39$, $p = 0.012$, and **b)** mean number of offspring per day. $R^2 = 0.30$, $F_{1,13} = 5.52$, $p = 0.035$. Data for female fecundity were square root transformed.

Grasshopper phenology

The phenology of *C. parallelus* in the study sites in 2007 was not related to the local plant species richness, i.e. there were no systematic differences in developmental speed between the study sites. When individual regressions were performed with percent grasshoppers in the respective developmental stage *versus* plant species richness in the study sites, plant species richness did not explain the variability in the abundances of any of the nymphal instars (nymphal stage 2: $F_{1,9} = 0.039$, $p = 0.848$, nymphal stage 3: $F_{1,9} = 1.203$, $p = 0.301$, nymphal stage 4: $F_{1,9} = 2.156$, $p = 0.176$, adults: $F_{1,9} = 0.050$, $p = 0.828$).

Discussion

The fitness of the grasshopper *C. parallelus*, measured as the number of egg pods laid by females, and by the number of hatching offspring, greatly varied between different grassland sites investigated. The variation in grasshopper fitness was best explained by plant species richness in the different study sites. This was true for both years (2005 and 2007) in which grasshopper fecundity was investigated. Furthermore, grasshopper abundances were positively affected by increasing plant species richness and, to a lesser extent, plant species composition in the study sites. Our results show that in addition to effects of plant diversity on specialist insect herbivore fitness, there are also measurable effects of plant diversity on the abundance of these generalist insect herbivores. We will discuss possible mechanisms underlying the observed patterns in female grasshopper fitness, in particular the role of a diverse diet for generalist herbivores.

Host specificity of C. parallelus

There is clear evidence from our feeding experiments the grasshopper *C. parallelus* is a true generalist feeder *sensu* Schoonhoven et al. (1998) that feeds on grass and legume species, but largely avoids the consumption of forbs. Feeding behaviour of acridid grasshoppers including *C. parallelus* in the field has been studied by observations and gut analyses (see ref. in Chapman & Joern, 1990). Differences between grasshopper species belonging to different species within one subfamily could be found with respect to the relative amount of grasses and forbs in their diet (Joern, 1979). The consumption of food plants was not exclusively determined by the abundance of food plant species in the grasshoppers' habitats (e.g. Bernays et al., 1970b), which is an indirect proof that grasshoppers exert active food choice.

Grasshopper abundance and fitness in relation to habitat variables

The abundances of *C. parallelus* in the field and female fitness were higher in sites with higher plant species richness and none of the other habitat parameters tested in our study was significantly correlated with grasshopper fitness. The abundances of *C. parallelus* were additionally influenced by plant community structure (NMDS) and by solar radiation in the sites. Plant community composition has already been shown to explain a large fraction of the variance in insect abundances (e.g. Perner et al., 2005). Plant community composition is a surrogate for a number of effects the plant community exerts on herbivorous insects that are difficult to identify, because they include effects on grasshopper diet (i.e. combinations of particularly suitable or unsuitable food plants), microclimatic effects and structural effects such as refuges from natural enemies (Perner et al., 2005). Our study sites are at elevations up to 685 m above sea level with high annual precipitation and low average temperatures. In grasshoppers, like in most ectothermic animals, both activity and growth are temperature-dependent. It is thus conceivable that sunshine is a major restriction for *C. parallelus* activity in the study area, which may explain why there are more grasshoppers in sites more exposed to sunshine. Data on the relative abundances of *C. parallelus* nymphal instars in the different study sites in 2007 show, however, that the phenology of the grasshoppers was not correlated with plant species richness. Thus, the positive effect of plant species richness on grasshopper fitness is not confounded by systematic differences in phenology, i.e. a systematic difference in female grasshopper age among sites at the time of capture.

The consistent effect of plant species richness on herbivore fitness and the absence of significant correlations with other habitat parameters suggest that the plant diversity *per se* has positively affected female nutrition, which resulted in the observed differences in fecundity and the number of offspring among sites. There are at least three possibilities for the positive affect of plant species richness on grasshopper performance: (1) an increase in the diversity of food plant resulting in a better diet, (2) a decrease in top-down control of grasshoppers by natural enemies with increasing plant species richness, and (3) a positive effect of plant species richness on other, unmeasured, abiotic variables that also positively influence grasshopper fitness. In our view, most evidence points to explanation (1), a positive effect of plant species richness on grasshopper fitness via the increased possibility of dietary mixing. Dietary mixing has repeatedly been shown to increase fitness in generalist grasshoppers including *C. parallelus* (e.g. Bernays et al., 1997; Bernays et al., 1994; Unsicker et al., 2008). Plant species richness and hence the number of potential food plants for *C. parallelus* doubled along the plant diversity gradient in this study. Although the results from the feeding experiment with *C. parallelus* show that this species predominantly feeds on grass and legume species, we observed

some feeding on other forbs. As we cannot exclude the possibility that feeding on these forbs affects grasshopper fitness despite the low amounts consumed, we used overall plant species richness in the statistical model presented here rather than species richness based only on grass and legume species. Multiple regressions using grass and legume species richness gave the same results for the number of grasshopper egg pods and grasshopper abundance, only the number of offspring was not significantly affected (data not shown). Experiments that tested feeding strategies in generalist grasshoppers to date have mostly tested a restricted number of plant species offered in monocultures or as mixtures and to our knowledge, only one study observed beneficial effects of a plant community containing more than 20 plant species in the field (Pfisterer et al., 2003). Pfisterer *et al.* found positive effects of plant species richness on weight gain of the generalist grasshopper *Parapleurus alliaceus* in a setting where grasshoppers were caged on experimental mixtures of one, two, four, eight and 32 plant species, all of which included at least one grass species. While the difference was mainly due to a higher weight gain in 32-species mixtures compared to mixtures of eight or fewer species, this study in an artificial grassland system also indicated positive effects of plant diversity on generalist herbivore performance. Interestingly, Specht et al. (2008) caged *C. parallelus* in artificial mixtures of 1-60 plant species half of which did not included grasses and found an overwhelming effect of grass presence on grasshopper fitness while the effect of plant species richness *per se* was not significant. While a cage experiment may not mimic natural conditions entirely, our correlative study can therefore not rule out additional effects of plant species richness on grasshopper fitness that are mediated by other factors than diet composition, i.e. possibilities (2) and (3). The risk of predation for grasshoppers, in particular by spiders, has been shown to greatly influence grasshopper foraging behaviour (e.g. Schmitz, 2003) with possible consequences for nutrition and fitness. Unsicker et al. (2006) found in a study in the same study area a general increase in spider abundances with increasing grasshopper abundances, suggesting that top-down control does not decrease with increasing plant species richness. While we do not have information on other predators such as passerine birds, it appears that the generally lower vegetation in the more species-rich meadows (Kahmen et al., 2005, Unsicker et al., 2006) would increase rather than decrease foraging success of birds. Similarly, we cannot exclude the possibility that other abiotic factors such as the structure of the vegetation favour grasshopper reproduction more in the more diverse sites. Overall, however, the results from our correlative study suggest that both grasshopper abundance and fitness are controlled bottom-up mediated by food plant availability.

On the other hand, there are also top-down effects of grasshoppers on the plant community itself. Grasshoppers are important components of arthropod assemblages in grassland ecosystems and it has already been shown experimentally that they can alter plant populations and community dynamics by e.g. suppressing abundant, highly competitive grass species and thus facilitating the evenness in

grasslands (e.g. Schmitz, 2003). Although we have no experimental evidence in our study sites, it is conceivable, that *C. parallelus* populations that mainly feed on grasses also affected plant species richness and plant community composition in the study sites. Grasshopper abundances are highest in species rich meadows and as these grasshoppers mainly feed on grasses and some legumes, it is likely that they prevent highly competitive grass species from dominating these sites. This in turn would benefit the competitively inferior forb species. Long-term grasshopper exclusions could shed light on the question whether bottom up forces shape grasshopper communities or whether grasshopper populations sustain high levels of plant diversity through top-down effects.

The results from our study imply that the fitness of polyphagous herbivores may be diminished in low diverse plant communities. Therefore, declines in biodiversity pose a threat to the persistence of generalist herbivores by inhibiting optimal feeding patterns.

Acknowledgments

Supplementary material

Table S1: Linear regressions for the grasses and forbs that were investigated in the grasshopper feeding experiment with respect to the two non-metric multidimensional scaling axes (NMDS). R^2 and p values are given. Marginally non significant results are also displayed. Arrows indicate the direction of the relationship.

		NMDS1 (R^2, *p*)	NMDS2 (R^2, *p*)
Grasses	*Agrostis tenuis*	n.s.	0.463, 0.005 ↑
	Anthoxanthum odoratum	n.s.	0.334, 0.024 ↑
	Dactylis glomerata	n.s.	0.612, 0.001 ↓
	Festuca rubra	n.s.	0.614, 0.001 ↑
	Holcus lanatus	n.s.	n.s.
	Phleum pratense	0.225, 0.074 ↑	n.s.
	Trisetum flavescens	0.372, 0.016 ↓	n.s.
Forbs	*Alchemilla vulgaris*	n.s.	0.204, 0.091 ↑
	Hypericum maculatum	n.s.	n.s.
	Plantago lanceolata	0.503, 0.003 ↓	n.s.
	Trifolium pratense	0.233, 0.068 ↓	n.s.
	Trifolium repens	n.s.	n.s.
	Veronica chamaedrys	n.s.	0.216, 0.081 ↑

Chapter II

Grasshopper fitness depends on parental experience

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Abstract

On the basis of a broad and diverse food plant spectra generalist insect herbivores are able to establish populations in meadow habitats characterized by different plant species diversity and composition. The positive influence of a broad diet on generalist insect herbivore fitness was revealed in several studies and proved in the field most likely due to the mechanism of dietary mixing. In this study the performance of different *Chorthippus parallelus* grasshopper populations was tested with respect to food plant species richness and composition available throughout grasshopper development. Three to four cages were installed in six meadows differing in plant species number and composition. The grasshoppers that were released in these cages were either caught in the same meadow (*home*) or were relocated from another grasshopper meadow (*away*).

Our results show that *home* grasshoppers in the cages did not perform better with respect to developmental time, survival and the number of eggpods per female than *away* grasshoppers. However our data show that characteristics in the parental habitats such as plant species richness and plant biomass significantly affected the performance of grasshopper offspring. These effects of parental experience were stronger than the actual site conditions (e.g. food plant species richness and biomass) the grasshoppers were reared under. This result might be a hint of strong maternal effects which is also discussed to be overlaid local adaptation in grasshopper populations of *Chorthippus parallelus.* Nevertheless, it is interesting how parent experiences are influencing the fitness of the subsequent generation even if this is a generalist insect herbivore and to be known as a very plastic feeder.

Keywords: *Chorthippus parallelus*, generalist insect herbivore, dietary mixing, plant species richness, local adaptation, maternal effects

Introduction

Abiotic (temperature, solar radiation) as well as biotic factors (plant species richness and composition) alone and in combination always characterize the habitats of herbivorous insects and thus directly and indirectly affect their abundance and performance.

Due to their diverse spectrum of food plants generalist insect herbivores, like grasshoppers are able to live on meadows featuring differences in meadow attributes like plant species richness and composition. An array of accepted food plants for generalist insect herbivores is accompanied by differences in food plant spectra, diversity and quality, which might lead to variations in herbivore performance among different meadow habitats. In this context, studies concerning performance differences in the grasshopper species *Chorthippus parallelus* and *Parapleurus alliaceus* among different meadows revealed therefore a positive relationship between plant species richness and insect herbivore performance (Pfisterer et al., 2003; Unsicker et al., 2010). This phenomenon is often based on the dietary mixing hypothesis which states that generalist insect herbivore performance profits of the possibility to switch among more or less suitable food plants for balancing nutrients or dilute toxins in the food (Bernays et al., 1992; Kaufmann, 1965; MacFarlane & Thorsteinson, 1980; Pulliam, 1975). A meadow habitat with higher food plant diversity is in this context assumed to contain more adequate food plants. Increased herbivore performance, mostly in grasshoppers, due to increased food plant richness was shown in different studies with real food plants which underlined the dietary mixing hypothesis (Bernays et al., 1994; Hagele & Rowell-Rahier, 1999; Miura & Ohsaki, 2004; Unsicker et al., 2008). Although Franzke et al. (2010) showed no performance differences in a generalist grasshopper species raised on different food plant compositions the study revealed differences in the conversion efficiency to body mass explained by selective dietary mixing which supports the active encounter of quality deficiencies in the food. A contradict result concerning the relationship between plant species richness and herbivore performance was shown by Specht et al. (2008) by caging fourth instar nymphs one experimental grassland communities along a diversity gradient, whereas the results indicates that grasshopper performance was more influenced by plant functional group identity than by plant species richness. Beside the contradictory results in several studies with the grasshopper species *Chorthippus parallelus*(Franzke et al., 2010; Specht et al., 2008; Unsicker et al., 2010; Unsicker et al., 2008) that were *inter alia* explained by differences in the experimental designs (laboratory vs. field studies, field caught vs. laboratory reared grasshoppers) it can be stated that varying food plant diversity, composition and quality in different meadow habitats can greatly influence the performance of generalist insect herbivores. However, Fox & Morrow (1981) points out that even a species of phytophagous insect that appear to be generalized in feeding habits, may actually be composed of populations locally specialized on different host species. Under these assumptions it might be possible that also generalized herbivore feeders are more or less adapted to

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certain meadows because of different meadow attributes, which are derived by diverse biotic and abiotic factors. In contrast to specialized insect herbivores which are often known to be locally adapted due to the limited range of food plants (Ballabeni et al., 2003; Hanks & Denno, 1994; Kuussaari et al., 2000; Zovi et al., 2008) the possibility of local adaptation in generalist insect herbivores to the complex arrangement of a meadow is rarely been examined (Parsons, 2011). Local adaptation to different environments is a central question in evolutionary biology and there has been a revival of interests in this question.

The aim of this study was to examine if the performance of grasshopper populations of *C. parallelus* differ as an expression of local adaptation to different meadows. The study organism has been proved to respond positively on plant species richness during a field observation (Unsicker et al., 2010) and is furthermore one of the most common grasshopper species throughout Central Europe (Ingrisch & Köhler, 1998; Köhler, 1981; Maas et al., 2002)and occurs in a variety of grassland types (Ingrisch & Köhler, 1998). To test local adaptation in *C. parallelus* a classic reciprocal feeding experiment was conducted (Kawecki & Ebert, 2004) with local vs. foreign (here *home* vs. *away*) grasshopper populations caged on six different *experimental meadows* in the field.

We asked three questions:

(1) Do *home* grasshopper populations exhibit a better performance than *away* grasshopper populations?

(2) How is the grasshopper performance influenced by originating from a meadow characterised with 'good' attributes in terms of e.g. plant species richness and vice versa (gradient between *meadow of origin* and *experimental meadow*)?

(3) To what extent are the grasshoppers influenced by the attributes of the *experimental meadow* they are caged on?

Material and Methods

Study organism and study area

Chorthippus parallelus (Zetterstedt) is one of the most abundant grasshopper species throughout Europe. This species is known as a generalist insect herbivore that preferentially feeds on grasses, but also a number of forbs and legumes (Bernays & Chapman, 1970; Franzke et al., 2010; Gangwere, 1961; Ingrisch & Köhler, 1998; Specht et al., 2008; Unsicker et al., 2008). *C. parallelus* is wingless and, like

most European grasshopper species, very territorial, which results in a low dispersal rate (Ingrisch & Köhler, 1998). The study area in the Franconian Forest in Central Germany is a low mountain rage at the border of Bavaria and Thuringia (50°21' – 50°34'N, 11°00- 11°37'E). The investigated meadows are part of a long-term biodiversity study along a gradient of plant species diversity (Unsicker et al., 2006). A main selection parameter for these meadows was extensive management for at least the last decade, including no fertilization, no grazing, a moderate mowing schedule of two cuts per year and a pH value of above 5.0.

Experimental design

Choice of the *experimental meadows* and the connected *away populations*

In order to test for local adaptation a classic *home* vs. *away* (explanation see below) experiment was designed (Kawecki & Ebert, 2004). Therefore six meadows were chosen along a gradient of plant species richness, referred as *experimental meadows*. On these six meadows grasshopper cages (1 per 1 square meter, covered with aluminum mesh at the sides and on the top) (see supplementary material **Picture 1**) were installed in order to cage the grasshopper population originated from this specific *experimental meadow* (*home* population) as well as two to three different grasshopper populations from other meadows (*meadows of origin*), which are referred as the *aways*. The *away* populations have been chosen depending on their largest differences from their *meadow of origin* in comparison to the *experimental meadow* they had been relocated. The criteria for this choice were (a) difference in plant species composition (b) difference in plant species richness and (c) the geographically distance between *experimental meadow* and *meadow of origin*(for detail see **Table 1**, second column – distinction in plant species richness, third column- distinction in plant species composition, fourth column – geographical distance)

To account for the effects of plant community composition *away* populations were chosen according a non-metric multidimensional scaling (NMDS) which has been produced during the analysis of grasshopper fitness experiment in the study area (see Unsicker et al. 2010, Chapter I). The difference in plant species richness had been calculated out of plant cover data which have been assessed within the BIOLOG project. Geographic distance between *experimental meadow* and *meadow of origin* has been calculated with Google Earth. As it was possible, due to different hatching success of the different grasshopper populations, the experiment was conducted with 4 cages on each of the six chosen *experimental meadows*. According the experimental design the 3 to 4 cages contained (1) the *home* grasshopper population (2) *away* population with distinction in its species composition regarding the *meadow of origin* (3) *away* population with distinction in its species richness regarding

the *meadow of origin* and if is it was possible (4) *away* population with distinction in geography between *experimental meadow* and *meadow of origin* (**Table 1**).

Table 1: Presentation of the experimental design with a list of all *experimental meadows* (first column) and the corresponding *away* populations (rows to each experimental meadow). In total 6 *home* populations and 15 *away* populations have been used in this experiment. The names of grasshopper populations are derived from the names which were given to the meadows in the BIOLOG DIVA project. Additionally, the table contains information about the number of caged individuals of *home* and *away* grasshopper populations per cage.

Experimental meadow	# grass-hoppers/ *home* pop.	*Away* pop. with distinction in species com-position	# grass-hoppers/ *away* pop.	*Away* pop. with distinction in species richness	# grass-hoppers/ *away* pop.	*Away* pop. with distinction in geography	# grass-hoppers/ *away* pop.
Schlegel -2	63	Tschirn -20	60	Tschirn - 23	60		
Oßla	50	Tschirn -22	50	Steinbach a.W.	54		
Tschirn -22	50	Teuschnitz -24	70	Steinbach a.W.	55		
Tschirn -23	57	Schlegel – 2	61	Tettau	35	Saaleaue Jena	64
Steinbach a.W.	59	Schlegel -1	54	Tschirn -20	62	Teuschnitz - 11	54
Saaleaue Jena	64	Teuschnitz – 13	43	Teuschnitz - 11	50	Schlegel - 2	61

To cover the greatest differences between the *home* and *away* populations with respect to the three just mentioned criteria (species composition, species diversity and geographically distance) the *away* populations have been placed on *experimental meadows* after a ranking. The experiment started between the 14th and 16th of July in 2006 and lasted over 3 month till the last females died in the laboratory at the 30th of October 2006. The grasshoppers of each population were hatched from eggs in the laboratory whereas the parental generation was caught in the field for reproduction in the laboratory one year before the experiment was conducted. The experiment started by caging the laboratory reared grasshoppers in the second larvae stage.

Grasshopper fitness parameters

Survival

After the start of the experiment the survival was assessed every second day and with the first individual became adult every day by counting the remaining grasshoppers in the cages. As it is known that grasshoppers preferentially are sitting in sunny places the cage was half-covered with a blanket to

lead all the grasshoppers to one side of the cage. To insure an exact counting the vegetation inside the cage was slightly touched with a bamboo stick, so that the grasshoppers jumped out of the vegetation onto the aluminum mesh of the cage. For every counting event the survival data were corrected in the following way: if at a given date the number of grasshoppers was higher than in a previous count, we assumed that individuals were overlooked in the previous count and adjusted the numbers of grasshoppers accordingly. After one week of maturity female grasshoppers had been transferred to the laboratory (see fecundity section) and survival was controlled every day, this fitness measurement is referred to as female survival lab.

Developmental time

As the first grasshopper (either male or female) in each cage became adult, counting took place every day to assure that the 3 first adult female and also the 3 first adult male for developmental time measurement purposes had been recorded. Regarding the fact that the hatching time was not the same for all individuals of the different grasshopper populations a personal developmental time was calculated for each of the 3 first adults (3 females, 3 males). This was done by taking the mean date of the hatching period of mostly 3 to 6 days into account.

Fecundity

Once the grasshopper female became adult, they were marked with a colour code which differed every day. Due to the colour coding it was possible to find the grasshoppers exactly one week after adult moulting in the cage to transfer them into the laboratory. In the meantime they had the possibility to freely mate in the cage in the field. In the laboratory, every female was held in a single fauna box (SAVIC, 27 x 18 x 18 cm) and were fed with the food plants from the *experimental meadows* they had been relocated on since the experiment started. In the box a pot with a sand soil mixture was offered to give them chance to lay eggs. After three weeks the pots were renewed in case the female were still alive. After the death of the females the pots were removed and the egg pods were counted.

Body mass

After the grasshoppers moulted into the third instar after releasing them into the cages six individuals of each cage were removed and frozen for body mass assessment. After drying for 24 hours with 70°C

they have been dry weighted. For the adult weight, the first three females and the first three males were captured right after moulting to dry weight them after the same procedure like described above.

Tibia and Femur length

The length of the tibia was measured for six nymphs and three males / three females which have been caught for body mass assessment. Before the grasshoppers were dried the tibia and femur length of each individual was measured with the help of a binocular using an object slide with a 0.1 mm scale.

Measurement of meadow attributes

In order to understand grasshopper response due to the relocation onto the *experimental meadows* so called *meadow attributes* have been measured on the *meadows of origin* and the *experimental meadows.* Therefore, *meadow attributes* are: plant species richness, plant species richness of grass, plant species richness of legumes, plant species richness of grass and legumes together, biomass, and also the fitness of the parental generation on each meadow. The difference between *original meadow* and *experimental meadow* in certain *meadow attributes* is called *Delta attribute* (see below, Statistical Analysis).

Aboveground biomass was sampled in four 0.1m ^{2} rectangles on each meadow (*experimental meadows* and *meadows of origin*) in 2005. This was done by cutting the vegetation 3cm aboveground, drying them at 70°C for 48h and weighed them. In visual cover estimation all plant species were identified in four 1x1 m quadrats on each of the *experimental meadows* and *meadows of origin* in 2005. Due to a fitness measurement on all meadows (all *meadows of origin* and all *experimental meadows* unless the Saaleaue Jena, see **Table S2**) in 2005 were females were caught and oviposition rates were measured it was possible to gain a parental fitness measurement with reference to the grasshopper populations used in this experiment (Unsicker et. al 2010). This *meadow attribute* is referred to as fitness parental generation.

Statistical Analyses

The data were analysed using IBM SPSS Statistics 19 for Windows and R-2.8.1. (R Development Core 2005). Analyses of variance (ANOVA) and linear mixed effect models (lme) were performed. Median survival time of grasshoppers was calculated from a Kaplan-Meier survivorship curve that was fitted separately for each cage in the experiment. Individuals still alive at the end of the experiment entered

the analysis as censored data. Individuals which died in the first four days after the experiment was started have been neglected within the survivorship curve, as the mortality directly after the start of the experiment was very high and is known to be often derived from handling. Grasshoppers which were caught for body mass measurement have also been ignored in the survivorship curve. The percentage of grasshopper individuals that reached maturity (referred to as percent adults) was calculated for each cage in the experiment. This was done using following formula:

total amount of adults per cage /100 * total amount of grasshopper individuals per cage at the beginning of the experiment.

Due to a continuous grasshopper counting in all cages over the whole time period of grasshopper development we were able to assess the percentage of individuals which reached the adult stage for every single cage. This is referred to as the percentage of adults. As not every cage contained the same number of females we calculated the number of egg pods per female as our measure of fecundity. Body mass gain (weight gain) was calculated out of the difference of body mass adult minus body mass in the third larvae stage. The start body mass (body mass in the third larvae stage) could not be separated after gender.

To describe the offspring fitness response of *away* grasshopper populations which were relocated on the *experimental meadows* that differed in certain attributes (plant species richness, fitness of parental generation etc.) as compared to the *meadow of origin* (meadow on which the parents have been caught)new variables were calculated. These independent variables are referred to as *Delta attributes* and were calculated in the following way: *Delta attribute*= attribute$_{meadow\ of\ origin}$− attribute$_{experimental\ meadow}$, whereas following attributes have been calculated: plant species richness, plant species richness grass, plant species richness legumes, plant species richness grass + legumes, biomass and fitness of parental generation (for details see **Table S1**). These calculations have been done for every *away* population and *home* population where in the latter case (home populations) the *Delta attribute* is zero as *experimental meadow* equals *meadow of origin*.

Analyses of variance (ANOVA) were performed to test for the effect of *home* vs. *away* and for the effect of meadow identity on grasshopper fitness. The full model used in this test was: Fitness measure ~ *experimental meadow* + treatment, where treatment defines either *home* or *away* population and *experimental meadow* defines the six different *experimental meadows* in the experiment. As the experiments contained more *away* than *home* populations for every *experimental meadow* a mean of *away* populations was calculated. It sometimes occurs that the full model indicated a relationship between the fitness measurement (e.g. survival, fecundity, development time) and one of the independent variable (either treatment or *experimental meadow*) which was expressed

as a marginal non significant result. After a model simplification the marginal non significance turned out to be a significant relationship. If the simplified model was shown to be non significantly different to the full model the simplification was accepted. Linear mixed effect models (LME) were performed to test grasshopper performance due to differences in *meadow attributes* between *meadow of origin* and *experimental meadows* (Deltas). The null model used in this test was: lmer (fitness measure ~ 1 + (1|*experimental meadow*) and the full model was: lmer (fitness measure ~ 1 + Delta + (1|*experimental meadow*). The statistical significant difference in the grasshopper fitness measurement derived by a *Delta attribute* was tested by the R command: anova (null model, full model).

Results

Meadow identity effects on grasshopper fitness

The grasshoppers laid significant different amounts of eggpods per female after feeding on the six different *experimental meadows* (eggpods per female: full model including treatment: $F_{5,4}$= 4.27, p = 0.09, simplified model: $F_{5,5}$ = 5.3, p = 0.045)(**Figure1**). Also the developmental time of female grasshoppers showed a marginal non significant trend to differ according to the six *experimental meadows* in the experiment (developmental time female: $F_{5,5}$ = 4.6, p = 0.06)(**Figure 1**). The number of eggpods per females and the developmental time of female grasshopper were therefore negatively correlated with each other (Regression: r^2= 0.32, $F_{1,19}$ = 8.3, p = 0.01)(see **Table 3**).
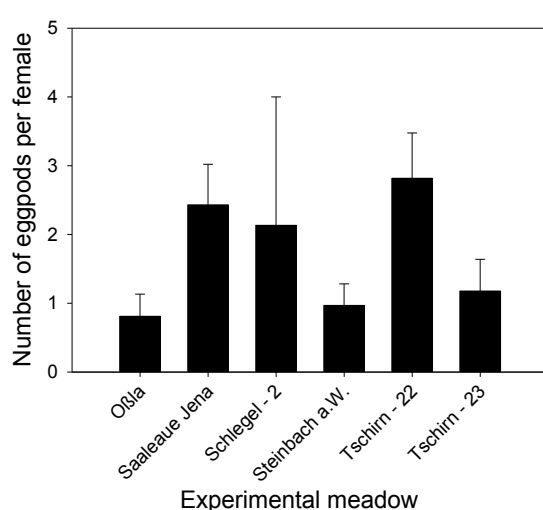
(a)   (b)
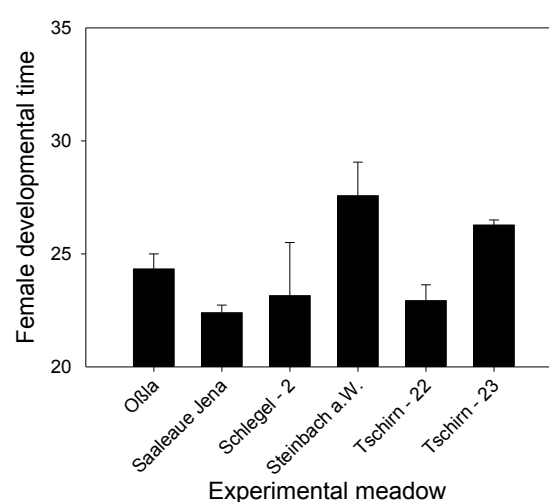
Number of eggpods per female (**a**) and female developmental time in days (**b**) in dependence of *experimental meadow* identity. A mean value over all cages regardless of grasshopper origin (*home* population and *away* populations) was therefore calculated for every *experimental meadow*. The names of the *experimental meadows* have been derived from the names which were given to the meadows in the BIOLOG DIVA project.

Grasshopper fitness differences between home and away populations

Away grasshopper populations showed a significant higher survival than *home* grasshopper populations which originated from these meadows (survival: $F_{1,5}$= 6.9, p = 0.046)(**Figure2**). Also the percentage of grasshopper individuals that reached the adult stage was significantly higher in *away* populations in comparison to *home* populations (percent adult: $F_{1,5}$= 8.5, p = 0.03) (**Figure 2**).
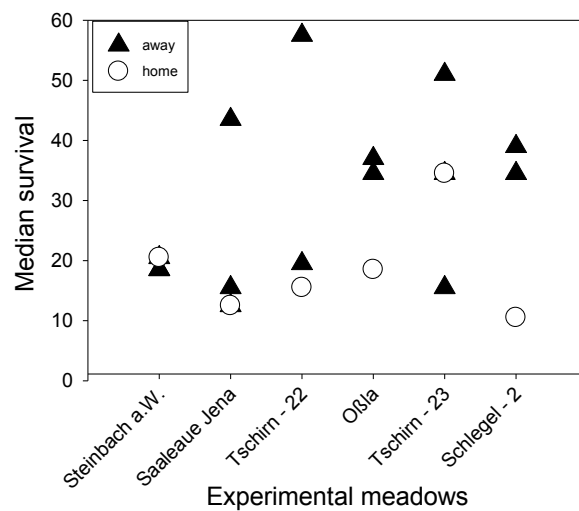
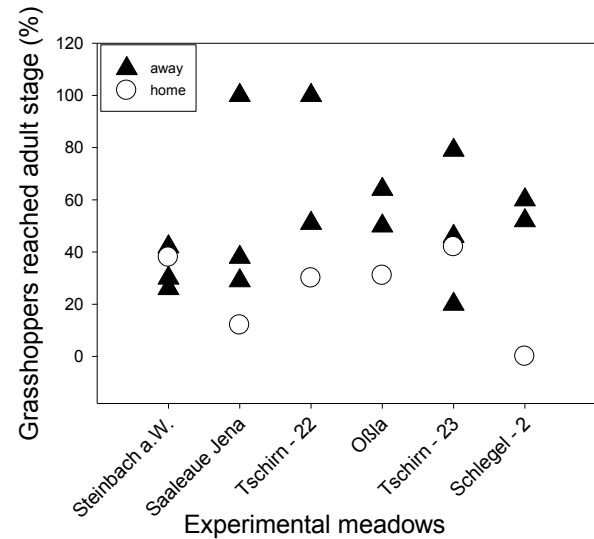(a)   (b)

Figure 2: Grasshopper survival in days (a) and for the percentage of grasshoppers that reached the adult stage (b) presented for *home* and *away* populations for each of the six *experimental meadows* (circles = *home* populations, triangle = *away* populations).The names of the *experimental meadows* have been derived from the names which were given to the meadows in the BIOLOG DIVA project.

Grasshopper performance in response to meadow specific attributes

Delta plant species richness

The developmental time of female grasshoppers was significantly prolonged in populations that originated from meadows (*meadow of origin*) with a lower plant species richness (mean developmental time female with lower plant species richness on *meadow of origin*: 25.8 ± 1.2 days, mean developmental female with higher plant species richness on *meadow of origin*: 23.7 ± 0.81 days) than the *experimental meadow* were they have been relocated to. The same trend, but marginal not significant, was revealed in male developmental time (**Table 2, Figure 2**).There was a marginal non

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significant trend resulting in a lower number of eggpods per female in grasshopper populations from *meadow of origins* with a lower plant species richness than the *experimental meadow* (**Table 2, Figure 2**). An increasing delta of plant species richness between *meadow of origin* and *experimental meadow* was not influencing grasshopper survival (**Table 2, Figure2**).

Delta species richness grass

Female developmental time was significantly prolonged in populations which originated from meadows (*meadow of origin*) with a lower grass species richness (mean developmental time female with lower grass species richness on *meadow of origin*: 27.3 ± 1.2 days, mean developmental time female with higher grass species richness on *meadow of origin*: 23.0 ±0.62 days) than the *experimental meadow* were they have been relocated on. The same trend but marginal not significant was true for the developmental time of male grasshopper populations (**Table 2**).

Delta species richness legumes

A difference in legume presence between *original* and *experimental meadow* was not influencing grasshopper performance except the weight gain of male grasshoppers. However, if the *meadow of origin* had a lower legume species richness than the *experimental meadow* the relocated grasshopper populations showed a non significant trend to lay fewer eggpods and also female survival decreased. On the contrary, male grasshoppers gained more weight if the *meadows of origin* showed a lower plant species richness of legumes than the *experimental meadow*.

Delta species richness legumes and grass

Female developmental time was significantly prolonged in populations which originated from meadows (*meadow of origin*) with a lower species richness of grasses and legumes (mean developmental time female with lower grass and legume species richness on *meadow of origin*: 26.7 ± 1.5 days, mean developmental time female with higher grass and legumes species richness on *meadow of origin*: 23.3 ± 0.7 days) than the *experimental meadow* were they have been relocated to. (**Table2**).

Delta biomass

Developmental time of each female and male grasshopper was prolonged if the *meadow of origin* features a higher biomass than the *experimental meadow* (Mean developmental time females higher biomass on *meadow of origin*: 27.2 ± 3.01 days, mean developmental time of females lower biomass on *meadow of origin*: 25.2 ±0.61 days). Along this finding grasshopper survival, the percentage of grasshoppers that reached the adult stage, number of eggpods per females and the survival of females in the lab was negatively influenced by a higher biomass of the *meadow of origin* (**Table 2, Figure 2**).

Delta fitness parental generation:

A low parental fitness, expressed through the number of eggpods per females in the parental generation (data derived from the study by Unsicker et al. 2010, see Chapter I) caused a significant prolonged female and male developmental time (mean developmental time females with lower parental fitness on *meadow of origin*: 28.5 ± 1.5 days, mean developmental time females with higher parental fitness on *meadow of origin*: 24.3 ± 0.5 days). These findings are underpinned by the decrease of grasshopper survival and survival of females as well as a lower percentage of grasshoppers that reached the adult stage and a lower number of eggpods per female in grasshopper populations originated from *original meadows* with a lower fitness of the parental generation (**Table 2, Figure 2**).

Grasshopper weight gain and tibia length for male and females were shown to be not influenced by any difference in *meadow attributes* (plant species richness, biomass) between their *meadow of origin* and the *experimental meadow* (see **Table 2**).

Table 2: Results from the linear mixed effect model (lme) testing the influence of *Delta attributes* of *experimental meadows* in comparison to *meadow of origin* on grasshopper fitness measurements. Therefore the *Delta attributes* were calculated as the difference between *original meadow* and *experimental meadow* in certain *meadow attributes* (Delta attribute= attribute$_{meadow\ of\ origin}$– attribute$_{experimental\ meadow}$).The null model was performed for every fitness measure and compared due to the Akaike Information Criterion (AIC) and P value with the full model testing a difference in the grasshopper fitness measurement derived by a Delta attribute. Significant results are presented in bold P values.
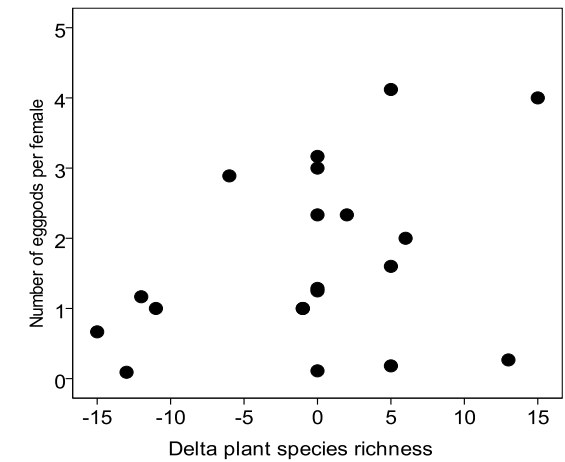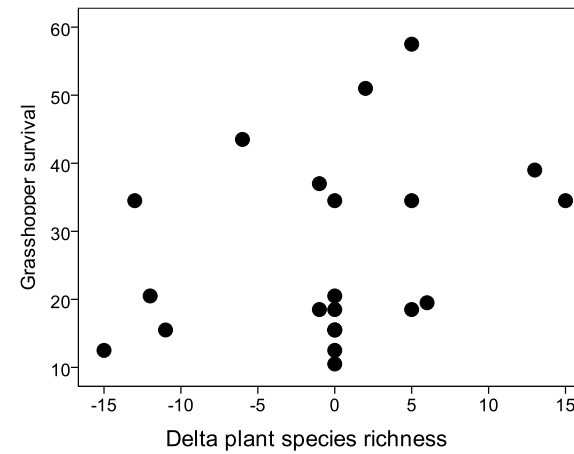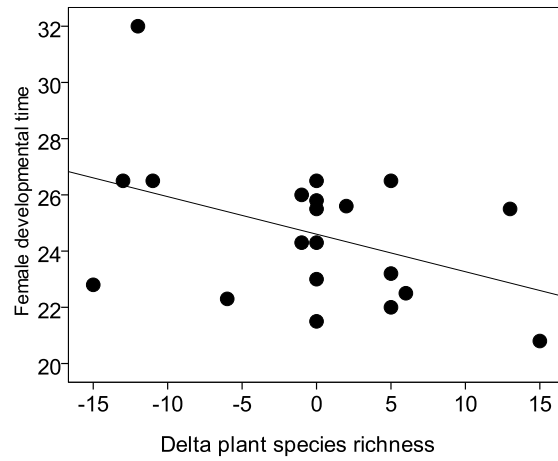
	DF	develop-mental time f		develop-mental time m		survival		grasshoppers that reached adult stage (%)		eggpods per female		survival female lab		tibia female		tibia male		weight gain male		weight gain female	
		AIC	P	AIC	P	AIC	P	AIC	P	AIC	P	AIC	P	AIC	P	AIC	P	AIC	P	AIC	P
Null Model	3	93.9		90.0		174.2		188.8		70.4		155.8		-77.4		-71.3		-185.6		-170.8	
Δ species richness	4	89.7	**0.012**	89.1	0.09	174.1	0.15	190.2	0.48	69.1	0.07	156.9	0.36	-77.1	0.18	-69.5	0.62	-185.6	0.16	-168.8	0.87
Δ species richness grass	4	87.1	**0.003**	89.1	0.09	175.9	0.6	189.6	0.28	71.1	0.26	157.2	0.44	-78.6	0.07	-69.8	0.5	-183.9	0.59	-170.4	0.19
Δ species richness legumes	4	93.3	0.1	91.9	0.99	173.8	0.12	190.8	0.99	69.6	0.09	154.6	0.07	-75.4	0.92	-69.4	0.73	-188.4	**0.03**	-171.4	0.10
Δ species richness legumes + grass	4	83.5	**<0.001**	89.2	0.09	174.5	0.19	189.6	0.28	70.3	0.15	157.8	0.86	-78.4	0.08	-69.6	0.59	-183.9	0.60	-168.8	0.76
Δ Bio-mass	4	72.8	**<0.001**	75.5	**<0.001**	147.4	**<0.001**	159.1	**<0.001**	61.0	**<0.001**	136.5	**<0.001**	-64.9	1	-57.6	1	-160.2	1	-161.5	1
Δ fitness parental generation	4	49.8	**<0.001**	69.9	**<0.001**	130.4	**<0.001**	147.3	**<0.001**	55.1	**<0.001**	121.1	**<0.001**	-60.8	1	-48.4	1	-144.4	1	-122.2	1

(a)Female Developmental time   **(b)Grasshopper survival**   **(c) Number of eggpods per female**

(a) Female Developmental time   **(b) Grasshopper survival**   **(c) Number of eggpods per female**

ΔFitness parental generation
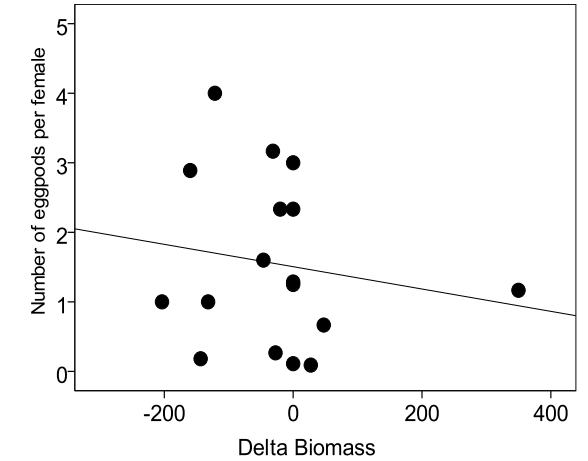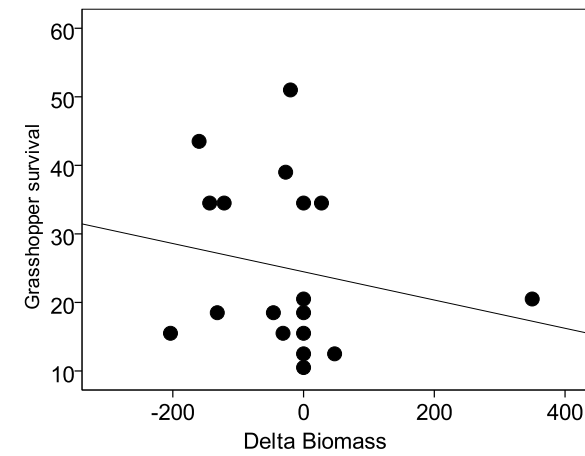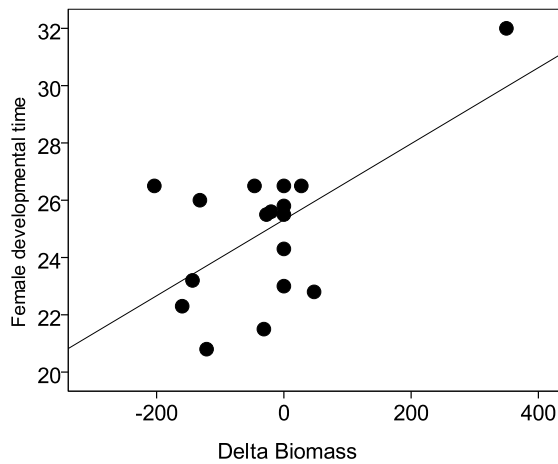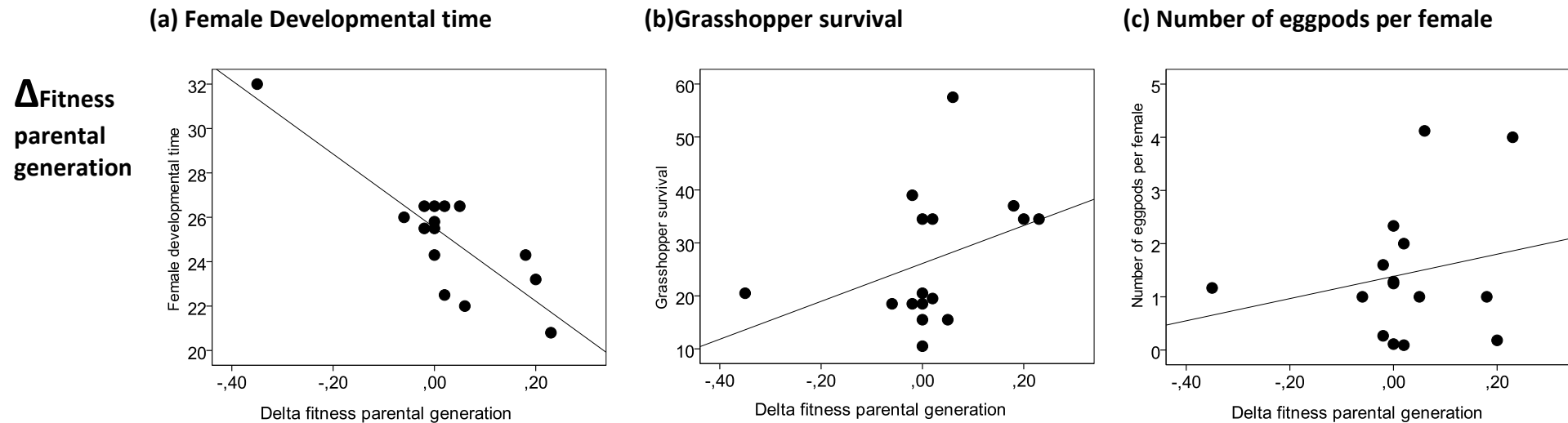
Figure 3: Female developmental time (a), grasshopper survival (b) and the number of eggpods per female (c) in dependence of Delta plant species richness, Delta Biomass and Delta fitness parental generation. Whereas, Delta attribute= attribute$_{meadow\ of\ origin}$− attribute$_{experimentalmeadow}$. According to this calculation a positive value on the x-axis indicates higher plant species richness (first row), higher biomass (second row) and a higher number of eggpods in the parental generation (third row) on the *meadow of origin* in comparison to the *experimental meadow* where *away* grasshopper populations have been relocated on.

Relationships between fitness measurements

With increasing grasshopper survival the percentage of grasshoppers reaching the adult stage increased (**Table 3**). Furthermore, the relationship between female survival and the numbers of eggpods per female was shown to be significant positive correlated with each other. With a prolonged developmental time of both female and male grasshoppers the number of eggpods per female was significantly decreased.

Table 3: List of correlations between fitness measurements (grasshopper survival, number of eggpods per female, percentage of grasshopper that reached the adult stage, female survival in the laboratory, male and female developmental time female of *Chorthippus parallelus*) whereas significant results are presented in bold letters ($p < 0.05$).

	total grass-hopper survival	number of eggpods	grasshoppers that reached adult stage (%)	survival female lab	develop-mental time female	develop-mental time male
total grasshopper survival		$r^2 = 0.06$, p = 0.302	$r^2 = 0.67$, **p< 0.001**	$r^2 = 0.026$, p = 0.49	$r^2 = 0.027$, p = 0.49	$r^2 = 0.018$, p = 0.557
number of eggpods			$r^2 = 0.03$, p = 0.43	$r^2 = 0.35$, **p = 0.006**	$r^2 = 0.240$, **p = 0.028**	$r^2 = 0.235$, **p = 0.033**
grasshoppers that reached adult stage (%)				$r^2 = 0.03$, p = 0.45	$r^2 = 0.05$, p = 0.34	$r^2 = 0.03$, p = 0.47
survival female lab					$r^2 = 0.03$, p = 0.45	

Discussion

In contrast to our hypothesis the results of the present study showed no local adaptation in grasshopper (meta-) populations of *C. parallelus* as *home* populations did not reveal a better performance than relocated *away* populations. It was furthermore discovered that grasshopper performance was increased by originating from meadows (*meadow of origin*) higher in plant species richness and lower in biomass. Additionally, a higher fitness in the parental generation leads to a

better performance in the observed grasshopper populations. In this case it was not of relevance if the parents lived on the meadow the grasshoppers were caged on (*home* populations) or the parents were living on another meadow *(away* populations) with different *meadow attributes*.

Undoubtedly, certain *experimental meadows* need to have attributes that caused higher grasshopper fitness than other *experimental meadows.* This was shown due to differences in mean grasshopper fitness per *experimental meadow* by taking the mean grasshopper fitness of both *home* and *away* populations together.

Grasshopper fitness and local adaptation

This study, however, revealed no local adaptation in grasshopper (meta-) populations of *C. parallelus* by living and feeding on their *meadows of origin*. Hence, this classical *home* versus *away* relocation experiment did not show a better fitness in *home* populations of *C. parallelus* compared to *away* grasshopper populations. This assumption was originally derived by the results of a study which pointed out a significant fitness difference between grasshopper populations due to a gradient of plant species richness on different meadows of the study area (Unsicker et al., 2010, see Chapter I). Nevertheless, local adaptation was yet mostly examined and detected mostly in monophagous insects on a broad geographically scale (Ballabeni et al., 2003; Hanks & Denno, 1994; Kuussaari et al., 2000; Zovi et al., 2008) and /or due to varying allelochemicals in the host plant as specialist insect herbivores often display adaptations to chemical defences such as tannins or glycosides (Reudler et al.; Zovi et al., 2008; Zvereva et al., 2010). However, one study that examined the effect of local food plant quality on the performance of the generalist grasshopper *Melanopus femurrubrum* found not always significant responses to diet but was otherwise again conducted along a broad geographically scale (Parsons, 2011). The lack of the broad geographic scale in the study area and the possibility of potential phenotypic plasticity due to feeding on different diets in *C. parallelus* proofed in the study by Franzke et al. (2010) might be responsible for not detecting local adaptation due to food plant species richness, composition and quality. In this relation it has to be discussed that local adaptation might be blurred by an unintentionally biased experimental design, as in certain *meadow attributes away* populations had an advantage due to good parental experiences. In these cases *away* grasshopper populations originated from meadows with better *meadow attribute*(s) (see **Table S2**). This was the case for *meadow attributes* like plant species richness grass, plant species richness legumes, plant species richness legumes and grass, biomass and the fitness of the parental generation (no data available for 4 populations (for detail see **Table S2**). In this context the *away* grasshopper populations

managed to maintain this fitness advantage in comparison to the *home* populations. This fact really supports the theory of maternal effects in this grasshopper species which is discussed in detail below.

Grasshopper fitness in response to specific meadow attributes

Anyway, grasshopper fitness in this study was positively responding on certain *meadow attributes* their parents experienced. This was proofed by the decreased developmental time of grasshoppers which parents originated from meadows richer in plant species of all plants and of grasses, as well as a decreased developmental time of grasshoppers in response to a higher species richness of legumes and grasses in the *meadows of origin*. As in this study developmental time was positively correlated with fecundity rate in female grasshoppers of *Chorthippus parallelus* it is not of a surprise that the fecundity was shown to be enhanced in females which originated from meadows with a higher plant species richness, although this trend was marginal not significant. Even though no general theory of how plant species richness might affect the abundance and fitness of generalist insect herbivores exists, in contrast to theories concerning specialists, the effect of dietary mixing in relation to increasing plant species diversity is often discussed in the literature (Unsicker et al., 2010, Chapter I). However, the dietary mixing hypothesis describes the theory that generalist insect herbivores broaden their diet by switching between complementary foods to balance nutrients and /or dilute toxins in the food (Bernays et al., 1992; Kaufmann, 1965; MacFarlane & Thorsteinson, 1980; Pulliam, 1975). An increase of plant species richness is therefore assumed to increase the repertory of food plant species (Unsicker et al., 2010, Chapter I). Studies with real food plants have been confirming the advantage of feeding on a diverse diet to increase herbivore fitness along the dietary mixing hypothesis (Bernays et al., 1994; Hagele & Rowell-Rahier, 1999; Miura & Ohsaki, 2004). Experimental observations with the study organism *C. parallelus* revealed a positive relationship between plant species diversity and grasshopper fecundity in the field (Unsicker et al., 2010, Chapter I) which was also discussed using the dietary mixing hypothesis. Anyhow, at the same time it was affirmed by Unsicker et al. (2010) that also possible additional abiotic effects which are mediated by increased plant species richness positively influences fitness of *C. parallelus* in the study area. This is underlined by the finding that a lower biomass on the *meadow of origin* leads to a decreased developmental time in female and male grasshoppers. Hence, less biomass on the *meadow of origin* positively influenced grasshopper survival and the total number of eggpods per females in the next generation. These findings are supporting the fitness response of grasshoppers on plant species richness, described above, as plant species richness is negatively correlated with vegetation height and therefore biomass in this study site (Kahmen et al., 2005; Unsicker et al., 2006; Unsicker et al., 2010). Vegetation structure which is derived by plant biomass is creating optimal microclimate conditions and is therefore a main driver for grasshopper

occurrence in central European grasslands (Ingrisch & Köhler, 1998). Branson et al. (2006) is arguing that many grasshopper species occur in microhabitats with bare soil and little shade, altogether less biomass, due to the enhanced capacity for thermoregulation in this microhabitat. The same could be applied for the ectothermic grasshopper species *C. parallelus* which occurs in our study site characterized by high annual precipitation and low average temperatures and might therefore be temperature dependent. Nevertheless, adequate temperatures for activity and growth of *C. parallelus* might be better reached in habitats with less biomass and hence higher solar radiation (Unsicker et al., 2010, Chapter I).

Grasshopper fitness in response to fitness of parental generation – maternal effects?

High fitness of parents, expressed through a better rate of eggpods per females in the parental generation (parents from *meadows of origin*) promises also a good performance in the next generation which was shown in a decreased developmental time of female and male grasshoppers, higher rate of grasshopper survival and grasshoppers which reached the adult stages as well as a greater number of eggpods produced per female. The study of Unsicker et al. (2010) described the circumstance under which grasshoppers of *C. parallelus* in the study area reached a high fitness which is among other things mainly depending upon a high plant species richness of the meadows. However, a good parental fitness derived by positive environmental aspects like food plant quality or in this case diversity could be an advantage for offspring *per se* and might be a consequence of so called maternal effects. In this context, maternal effects are described as the influence of parental environment which leads to the transfer of information from maternal generation to the phenotype of the offspring, whereas the maternal effects do not result in a genetic change of the offspring (Hunter, 2002; Mousseau & Fox, 1998). Thus, various attributes can serve as parental environment whereas climate, food quality, food diversity and predation pressure may only serve as some examples in this relation (Hunter, 2002). Indeed, food plant quality like the allelochemistry of the parental diet in larvae of the tobacco budworm *Heliothis virescens* or the lack of essential nutrients in the parental diet of the Lepidoptera *Plutella xylostella* are shown to be influencing the offspring performance and development (Behmer & Grebenok, 1998; Gould, 1988; Hunter, 2002). However, given this background maternal effects could be an adequate explanation for the better fitness of grasshoppers which have been originated from meadows with a better parental fitness, although this experimental design do not allow a straight statement about maternal effects.

To conclude:

It was mostly revealed that offspring gained a fitness advantage by parents who experienced more plant species and less biomass in the *meadows of origin*. In this context grasshopper fitness was increased if parents originated from a meadow which had better *meadow attributes* than the *experimental meadow*. It was also of advantage if the parents had a better fitness *per se*, which is proved to be (in-) directly influenced by plant species diversity in the study area (Unsicker et al., 2010, Chapter I).Anyway, the most interesting discover were the probability of maternal effects in this species which might also overlaid a local adaptation. However, even as *C. parallelus* populations in this study area are proved to be not genetically different between the meadow habitats (Wiesner et al., 2011) the present examination nevertheless gives a hint of active maternal effects which has to be approved in further experiments.

Acknowledgements

Supplementary material

Table S1: List of *Delta attributes* measured in 2005 one year before the experiment was started on all meadows where grasshopper populations were sampled for this experiment. The names of the meadows are according to the meadow names are given in the BIOLOG DIVA project.

Delta attribute	Populations											
	Schlegel-1	Schlegel-2	Oßla	Teuschnitz-11	Teuschnitz-13	Steinbach a.W.	Tettau	Tschirn-20	Tschirn-22	Tschirn-23	Teuschnitz-24	Saaleaue Jena
plant species richness	X	x	x	x	x	x	X	x	x	x	x	x
plant species richness grass	X	x	x	x	x	x	X	x	x	x	x	x
plant species richness legumes	X	x	x	x	x	x	X	x	x	x	x	x
plant species richness legumes + grass	X	x	x	x	x	x	X	x	x	x	x	x
Biomass	X	x	x	x	x	x	X	x	-	x	x	x
fitness parental generation	X	x	x	x	x	x	X	x	x	x	x	-

Table S2: Presentation of the distribution of *away* populations according to the differences in *meadow attributes* between *experimental meadow* and *meadow of origin* in the experiment. Unless biomass grasshopper fitness was always increased if they have been relocated from a higher to a lower *meadow attribute*. The *meadow attributes* are in detail: plant species richness, plant species richness grass, plant species richness legumes, plant species richness legumes and grass, biomass, fitness of parental generation.

Meadow attribute	Number of *away* populations relocated from lower to higher *meadow attribute*	Number of *away* populations relocated from higher to lower *meadow attribute*	Number of *away* populations with the same *meadow attribute* between original and *experimental meadow*	Number of *away* populations with no information
plant species richness	7	7	1	-
plant species richness grass	5	9	1	-
plant species richness legumes	6	7	2	-
plant species richness legumes + grass	5	8	2	-
Biomass	9	3		3
LAI	9	5	1	
fitness parental generation	4	7		4

Picture 1: Grasshopper cages on one of the six *experimental meadows*, whereas a single cage had a size of 1 per 1 square meter and was covered with aluminium mesh at the sides and on the top.

Chapter III

Rearing conditions affects grasshopper response to food plant diversity

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Abstract

Generalist insect herbivores inhabit environments differing in host plant species richness and composition and thus they need to cope with varying host plant composition and quality. Dietary mixing has been suggested as a mechanism to overcome unbalanced diets. For the grasshopper *Chorthippus parallelus* it was in a laboratory experiment previously shown that individuals reared on a broader diet was beneficial yet this effect disappeared when grasshoppers were given access to the full range of host plants from their original habitat. It was also suggested that early feeding experience in the field apparently affected the effects of diet on performance later in grasshopper development. We reared *C. parallelus* individuals from different meadow populations, either caught as late instars in the field, or reared in the lab, with a *standard diet* consisting of two food plants or on a diverse *meadow diet* consisting of the food plants harvested in the grasshoppers` original habitats.

Survival and fecundity was higher in *laboratory-reared* than in *field-caught* grasshoppers but the effect of diet on performance depended on grasshopper origin. While *laboratory-reared* grasshoppers had higher survival and fecundity when fed on the *meadow diet,* diet had no effect on fitness in *field-caught* grasshopper. C/N-ratios were lower in the *standard diet* than in the *meadow diet*, but a high N-content of host plants did not result in higher overall fitness.
Our results emphasize the importance of dietary mixing for the performance of generalist herbivores but also stress that early larval experience including feeding experience is an important determinant of diet effects on later grasshopper developmental stages.

Introduction

Due to their broad feeding spectrum generalist insect herbivores can occur in habitats differing greatly in host plant species richness and composition. Although many studies underline the effect of differences in host plant availability and quality for herbivore performance (Hanski & Singer, 2001; Singer & Thomas, 1996) research in this field of ecology has so far mainly focused on specialist herbivores (Mody et al., 2007; Warbrick-Smith et al., 2009). Studies on the performance of generalists occurring in different habitats are scarce (Tscharntke et al., 2002, Franzke et al, 2010). Nevertheless, generalist grasshoppers have been shown to cope with highly variable host plant quality (e.g. Bernays et al., 1994; Bernays & Chapman, 1970a, b; Berner et al., 2005; Miura & Ohsaki, 2004; Raubenheimer & Jones, 2006; Raubenheimer & Simpson, 2003; Simpson & Raubenheimer, 2000; Simpson et al., 2004; Unsicker et al., 2008). For example, the grasshoppers *Locusta migratoria* and *Schistocerca gregaria* are able to balance their intake of the two macronutrients nitrogen and carbon from nutritionally complementary synthetic food (Raubenheimer & Jones, 2006). A number of studies have shown that generalist insect herbivores also perform better on a mixed diet consisting of several plant species than on single host plants (Bernays & Bright, 1993; Bernays et al., 1994; Bernays & Minkenberg, 1997; Miura & Ohsaki, 2004; Unsicker et al., 2008). Dietary mixing may allow generalist herbivores to compensate for differences in plant species richness and composition among habitats especially if nutrients are unevenly distributed among plants, both in space and time (Behmer et al., 2002; Gusewell & Koerselman, 2002; Joern & Behmer, 1998; Oleksyn et al., 2002; Osier & Lindroth, 2001; Raubenheimer & Simpson, 2003; von Fircks et al., 2001; Westoby, 1978). Feeding on a broader spectrum of food plants should generally increase the probability of obtaining well- balanced intake of macronutrients.

The grasshopper *Chorthippus parallelus*, one of the most abundant grasshopper species in Central European grasslands (Ingrisch & Köhler, 1998), showed higher abundance and better performance (fecundity) in sites with high plant species richness as compared to habitats poor in food plants (Unsicker et al. 2010, Chapter I). A laboratory feeding experiment with *C. parallelus* individuals showed that grasshopper survival and fecundity was highest in the most diverse host plant mixture consisting of eight plant species offered and lowest in treatments with only single host plants (Unsicker et al. 2008). This study clearly emphasizes the positive effects of dietary mixing for this generalist herbivore and also revealed a strong dependency of food plant choice on grasshopper developmental stage. Yet in another experiment by Franzke et al. (2010) where *C. parallelus* individuals from one population were offered different food plant mixtures containing 11 to 15 plant species, no effects of diet composition and species richness in the diet on grasshopper performance were detected (Franzke et al., 2010). By transferring fourth instar nymphs of field caught individuals of *C. parallelus* into cages on

81 experimental grassland communities in plots containing one to 60 plant species neither plant species richness nor was plant functional group richness affecting grasshopper performance (Specht et al., 2008). The study by Specht et al. (2008) indicates that, at least in the field, grasshopper performance is more influenced by plant functional group identity, i.e. the presence of grasses, than by plant species richness *per se*.

These examples from studies on *C. parallelus* performance seem rather contradictory as in two of them food plant species richness had positive effects on grasshopper fitness (Unsicker et al., 2008; Unsicker et al., 2010, see Chapter I), whereas in the study by Franzke et al. (2010) there were no measurable effects of food plant number and composition on grasshopper performance. Because in Unsicker et al. (2008) only mixtures of maximum eight food plants were offered throughout grasshopper lifetime it is conceivable that beneficial effects through dietary mixing in grasshoppers are only evident below a certain threshold number of available food plants throughout grasshopper ontogeny. There are, however, a number of further differences between the studies. One major difference is that in Specht et al. (2008) grasshoppers were caught in the field before subjected to the experimental treatments whereas in Unsicker et al. (2008) effects of plant species richness were found in individuals reared from eggs in the laboratory. The early experience of nymphs in the field may therefore have influenced subsequent results.

The aim of this study was to investigate how diet composition (broad vs. narrow diet), and early experience (*laboratory-reared* vs. *field-caught*) affect fitness of the generalist grasshopper *C. parallelus*. We addressed the following questions:

(1) Do *field-caught* grasshoppers differ from *laboratory-reared* grasshoppers in the effect of diet on performance?

(2) Does a broad diet always result in higher fitness?

(3) Can *C. parallelus* nitrogen utilization explain the variation in grasshopper performance?

Material and Methods

Study organism and study area

Chorthippus parallelus (Zetterstedt) is an abundant grasshopper species occurring in different types of habitats throughout Europe. Together with other generalist grasshopper species, *C. parallelus* consumes approximately 0.2 to 4 % of plant biomass in European grassland ecosystems and thus plays an important role as a primary consumer (Ingrisch & Köhler, 1998). Although *C. paralellus* preferentially feeds on grasses, a number of forbs and legumes have also been reported as food plants in the literature (Bernays & Chapman, 1970b; Franzke et al., 2010; Gangwere, 1961; Ingrisch & Köhler, 1998; Specht et al., 2008; Unsicker et al., 2008). The study area in the Franconian Forest in Central Germany is a low mountain rage at the border of Bavaria and Thuringia (50°21′ − 50°34′N, 11°00-11°37′E). The investigated meadows are part of a long-term biodiversity study along a gradient of plant species diversity (Unsicker et al., 2006). A main selection parameter for these meadows was extensive management for at least the last decade, including no fertilization, no grazing, a moderate mowing schedule of two cuts per year and a pH value of above 5.0.

Laboratory-reared grasshopper experiment

We used grasshopper individuals hatched from eggs laid by females caught in 10 different meadows (10 grasshopper populations) in the study area (**Table 1**, **Table S1**supplementary material). To obtain eggs, 15- 20 adult female and 5- 10 adult male grasshoppers from the populations were caught with sweep-nets, between August and September of 2006. The grasshoppers were kept in cages (30 x 30 x 30 cm, MegaView Science Co., Ltd., Taiwan) for mating and oviposition in the lab for about four weeks. Throughout this time, all grasshoppers were fed *ad libitum* with the grass *Dactylis glomerata.* To allow oviposition, females were provided with pots filled with most sand-soil-mixture (1:3). The egg pods laid were kept in a fridge at 5°C over winter to simulate diapause. The pots with egg pods were watered periodically to prevent desiccation. In June 2007 all egg pods were removed from the fridge to allow grasshopper hatching. The progeny of each grasshopper population was kept separately. Freshly hatched nymphs were fed with *Dactylis glomerata*.

Table 1: Design of the experiment with *laboratory-reared* grasshoppers and *field caught* grasshoppers from different populations. The population numbers are according to the meadow numbers given in the Biolog DIVA project (see supplementary material Table S1). The table shows the numbers of grasshoppers within each cage per diet (*standard diet* vs. *meadow diet*). For population two no grasshoppers in the field could be caught and populations 11 and 21 did not exist as *laboratory-reared* grasshoppers.

Laboratory-reared grasshoppers			*Field-caught* grasshoppers		
population	grasshoppers on *meadow diet* cage 1 / cage 2	grasshoppers on *standard diet* cage 1/ cage 2	population	grasshoppers on *meadow diet* cage 1 / cage 2	grasshoppers on *standard diet* cage 1 / cage 2
1	6 /10	9 /10	1		
2	17 /22	17 /21	2	16	28
3			3	11 /13	9 /9
4	17 /20	15 /16	4	22	23
5	11 /11	13 /14	5	17	19
6	6	5	6	14	10
7	15 /15	12/15	7	11	10
8			8	7 /14	3 /14
9	13	10	9	10	14
10	9 /10	11/11	10	18	20
11	14 /16	12 /12	11	15	19
12	9/12	7 /10	12	14	10

The experiment was carried out in identical cages. The offspring of each population was divided into groups of 10 to 20 individuals per cage (**Table 1**). Hatchlings from one egg pod were distributed randomly across the two different feeding treatments (*standard diet* and *meadow diet*) (see **Table 1**). One or two cages were set up for each population and feeding treatment, depending on the availability of grasshoppers. The experiment with the *laboratory-reared* grasshoppers started when the grasshoppers reached the second instar by July 3rd 2007 and lasted over a three month period until the 16th of October 2007.

Field-caught grasshopper experiment

In early July, third and fourth instar grasshoppers were caught in eleven of the field sites in the study area(supplementary material, **Table S1**) (*field-caught* grasshoppers) by sweep netting. These grasshoppers were transported into the lab and directly placed in cages for the experiment (see **Table 1**). Depending on the available number of grasshoppers, one or two cages were set up for each

population and feeding treatment (*standard diet* and *meadow diet*). In the lab the *field-caught* grasshoppers were reared from the 18th of July 2007 over a 3 month period until the 16th of October.

Host plant selection and feeding experiment

Individuals in a particular cage were fed with either (**a**) a *meadow diet* that consisted of the most abundant host plant species in the grasshoppers original habitat or (**b**) a *standard diet* which comprised the grass species *D. glomerata* and the legume *Trifolium pratense*. In previous feeding experiments with *C. parallelus* both of these plant species were favoured by *C. parallelus* and positively influenced performance (Unsicker et al. 2008). Host plant selection concerning the *meadow diet* was based on vegetation surveys performed in 2005 and 2007 on the meadows in the study area. Mean plant cover abundances were calculated for all grass and legume species and all species that reached >1% in abundance in the respective meadow were selected as host plants (**Table 2**). Grasshopper host plants were collected weekly in the study sites and stored in plastic bags in a fridge at 5 °C. In the same manner the two food plants for the *standard diet* were sampled weekly on a meadow in the area of Jena city. The host plant species were offered separately in tubes of 3 cm x 2 cm x 2 cm filled with water in the same manner for both feeding treatments. The grasshoppers were fed twice a week. Food was provided *ad libitum*. All cages were set up in a laboratory at ambient temperature. To control for abiotic gradients in the laboratory setting (mainly light and temperature), differentially affecting the grasshoppers, cages with grasshoppers from the same *origin* (*laboratory-reared* or *field- caught*) but fed on different diets (*standard diet* vs. *meadow diet*) were arranged together in blocks.

Fitness measures

Survival

All grasshoppers in the experiment were counted every 3 – 5 days. Dead grasshoppers were individually transferred to vials and frozen. For every counting event the survival data were corrected in the following way: if at a given date the number of grasshoppers was higher than in a previous count, we assumed that individuals were overlooked in the previous count and adjusted the numbers of grasshoppers accordingly. As *laboratory-reared* and *field –caught* grasshoppers spent a different time until maturity in the lab we calculated adult survival which only comprises individuals that survived until adulthood.

Fecundity

For oviposition each cage was equipped with a plastic pot filled with an autoclaved moist sand/garden soil mixture (1:3). After the experiment was terminated, these pots were removed and the sand was sieved to count the number of egg pods.

Carbon and nitrogen contents in food plants, grasshoppers, and faeces

To determine nitrogen availability in the different diets and the consequences for grasshopper nutrient utilization, C/N analysis of plants, grasshopper bodies and grasshopper faeces were conducted. Five to seven individuals from each host plant species of each *meadow diet* and both host plant species of the *standard diet*, at least three up to 25 adult grasshoppers of each cage and the grasshopper faeces out of each cage were lyophilized. Male and female grasshoppers were analyzed separately. Both food plants and faeces were sampled at the same time to prevent variations due to the change of C/N ratio in plants during the season. Freeze-dried material was ground with a ball mill and dried in the oven for 24 hours at 70°C shortly before the C and N contents were analysed. C and N contents were analyzed for total carbon and nitrogen by dry combustion with a C/N analyser "Vario max" (Elementar Analysensysteme GmbH, Hanau, Germany). Total carbon and nitrogen in host plants, grasshopper bodies and grasshopper faeces are expressed as C/N ratios. Grasshopper body C/N was analyzed by taking the mean of all adult individuals over the cages belonging to the same population, diet (*standard* vs. *meadow diet*) and origin (*laboratory-reared* or *field caught*). As for the most *field-caught* grasshopper population only one cage per population and diet treatment existed (**Table 1**) the C/N analysis of grasshopper bodies and grasshopper faeces consists only out of one measurement point per diet treatment. The mean C/N ratio value was calculated for every population which was presented twice in the experiment per diet (**Table 1**). Males and females were analyzed separately.

C and N contents of grasshopper faeces were analysed for every single cage. The C/N ratio which remains in the grasshopper bodies (C/N turnover) was calculated for each cage in the following way:

$$\text{C/N ratio}_{diet} - \text{C/N ratio}_{faeces}$$

out of the difference between the C/N ratio of diet minus the C/N ratio of grasshopper faeces.

Table 2: List of grasshopper food plants offered to the different grasshopper populations in the lab (g= grass, l = legume). All grasses and legumes which had an abundance of >1 % on the specific meadows in the study area have been used for the feeding experiment. The plant abundances have been calculated out of vegetation estimations between 2005-2007 within the BIOLOG Diva project.

food plant	grass / legume	Meadow 1	2	3	4	5	6	7	8	9	10	11	12
Agrostis capillaris	g			x	X	x	x	x	X	x	x	x	x
Anthoxanthum odoratum	g			x	X	x		x	X		x	x	
Arrhenaterum elatius	g					x			X	x	x	x	x
Bromus hordeaceus	g												x
Cynosorus cristatus	g			x						x			
Dactylis glomerata	g	x	x	x	X	x			X	x	x	x	x
Elytrigia repens	g		x										x
Festuca rubra	g			x	X	x	x	x	X		x	x	x
Holcus lanatus	g				X								
Holcus mollis	g			x			x	x				x	
Lolium multiflorum	g	x											
Lolium perenne	g	x							X	x			
Luzula campestris	g			x			x	x					
Phleum pratense	g	x	x			x			X	x	x	x	x
Poa pratense	g	x	x			x					x		
Trisetum flavescens	g			x	X	x			X	x	x	x	
Lathyrus linifolius	l							x					
Trifolium pratense	l	x		x	X						x		
Trifolium repens	l	x	x	x				x			x		x
Vicia sepium	l											x	
plants total		7	5	9	7	8	4	7	8	7	10	9	8
grasses total		5	4	8	6	8	4	5	8	7	8	8	7
legumes total		2	1	1	1	0	0	2	0	0	2	1	1

Statistical analysis

The data were analysed using SPSS 15.0 for Windows and R-2.8.1. (R Development Core 2005). Regression analyses, analyses of variance (ANOVA) and correlations were performed. Assumptions of normality and heteroscedasticity were tested.

The *meadow diets* represented the field condition very well, as food plant richness in the experiment was positive correlated with the field site plant species richness ($F_{1,29}$ = 11.56, p = 0.002, r^2 = 0.28). Median survival time of grasshoppers was calculated from a Kaplan Meier survivorship curve that was fitted separately for each cage in the experiment. Individuals still alive at the end of the experiment entered the analysis as censored data. As not every cage contained the same number of females we calculated the number of egg pods per female as our measure of fecundity.

Analyses of variance (ANOVA) were performed to test for significant differences in grasshopper fitness (survival and fecundity) and the C/N ratios of plant, grasshopper and faeces. The standard model used in the tests was fitness measure ~ population*diet. Therefore it was tested if the variables of grasshopper fitness (survival and fecundity) and C/N ratios depended significantly upon diet effects (*standard diet* vs. *meadow diet*) or on population (different grasshopper populations in the experiment) effects. Tests were performed separately for laboratory and field treatment, as the effect of *origin* was very strong. Hence overall, 62 cages were used in the analysis and for analysis of treatment effects (diet) an average was taken whenever there were two cages for a particular treatment. The overall number of replicates was 36 cages of 10 populations for the test of diet on *laboratory-reared* grasshopper and replicates of 26 cages of 11 populations in *field- caught* grasshoppers (see **Table 1**).

An overall test including both of *laboratory-reared* and *field-caught* grasshopper populations revealed a dominant effect of grasshopper *origin* (*laboratory-reared /field- caught*). Only populations which are represented in both *origins* were used for this test (**Table 1**, 9 populations, 50 cages)

Results

Grasshopper performance

Laboratory- reared grasshoppers

Grasshoppers that were reared on *meadow diet* showed enhanced adult survival, male and female survival as well as female fecundity compared to the grasshoppers reared on *standard diet* consisting only of two food plant species (all grasshoppers: $F_{1,9} = 7.6$, p = 0.02; females: $F_{1,8} = 5.0$, p= 0.05; males: $F_{1,9} = 6.0$, p = 0.04; fecundity: $F_{1,8} = 6.5$, p = 0.03, **Table 3, Figure 1 a-b**). The ten grasshopper populations of laboratory reared grasshoppers showed no significant differences in adult, female, and male survival as well as in fecundity (all grasshoppers: $F_{9,9} = 1.2$, p = 0.4; female survival: $F_{9,9} = 0.9$, p = 0.5; male survival: $F_{9,9} = 0.8$, p = 0.6; fecundity: $F_{9,8} = 1.8$, p = 0.2). There was no significant interaction between diet and population (all grasshoppers: $F_{9,16} = 1.0$, p = 0.45; female survival: $F_{8,16} = 1.3$, p = 0.3; male survival: $F_{9,15} = 0.4$, p = 0.9; fecundity: $F_{8,15} = 1.3$, p = 0.3) and females and males did not differ in survival when feeding on the same diet (*standard diet*: df =1, $F_{1,33} = 1.57$, p = 0.22; *meadow diet*: df = 1, $F_{1,33} = 1.4$, p = 0.24; Table 3).

Field-caught grasshoppers

In contrast to the *laboratory-reared* grasshoppers, *field-caught* grasshoppers did not exhibit significant differences in survival when they were fed on *meadow* or *standard diet* (all grasshoppers: $F_{1,10} = 0.2$, p= 0.3, females: $F_{1,9} = 0.005$, p= 0.9; males: $F_{1,10} = 0.6$, p= 0.4; **Table3**, **Figure 1a**). In *field–caught* female grasshoppers, no effect of diet on fecundity was measurable ($F_{1,7} = 0.2$, p = 0.7, **Table 3, Figure 1b**). There were no significant fitness differences between the eleven populations of *field-caught* grasshoppers (all grasshoppers: $F_{10,10} = 1.8$, p = 0.2; female survival: $F_{10,9} = 1.2$, p = 0.4; male survival: $F_{10,10} = 1.6$, p = 0.2; fecundity: $F_{9,7} = 0.5$, p = 0.8). Females and males did not differ in survival by feeding on the same diet (*standard diet* df =1, $F_{1,23} = 0.003$, p = 0.96; *meadow diet* df = 1, $F_{1,22} = 0.99$, p = 0.33, **Table 3**).

Table 3: Performance of grasshoppers feeding on *standard diet* vs. feeding on *meadow diet* with respect to grasshopper *origin* (*laboratory-reared* grasshoppers/ *field-caught* grasshoppers). Mean ± SE.

	Laboratory-reared grasshoppers		*Field-caught* grasshoppers	
	standard diet	*meadow diet*	*standard diet*	*meadow diet*
adult survival (days)	45.4 ± 3.7	58.4 ± 3.2	34.8 ± 5.4	28.4 ± 4.4
female survival (days)	46.4 ± 4.5	59.7 ± 3.4	31.3 ± 4.6	30.3 ± 5.8
male survival (days)	38.9 ± 3.9	53.2 ± 4.3	31.6 ± 5.7	22.5 ± 5.1
fecundity (number of egg pods per female)	1.1 ± 0.2	2.5 ± 0.4	1.2 ± 0.4	1.6 ± 0.5
body C/N male	3.57 ± 0.03	3.75 ± 0.02	3.59 ± 0.04	3.7 ± 0.02
body C/N female	3.73 ± 0.03	3.94 ± 0.03	3.72 ± 0.08	3.97 ± 0.03
C/N grasshopper faeces	13.5 ± 0.2	17.6 ± 0.6	13.4 ± 0.3	18.2 ± 0.7
C/N plants - C/N faeces	-4.01	-1.35	-3.7	-1.79

Laboratory-reared vs. field-caught grasshoppers

Grasshopper *origin* was, however, important for survival (all grasshoppers: $F_{1,9}$ = 25, p < 0.001; females: $F_{1,7}$ = 21.3, p = 0.002; males $F_{1,9}$ = 15.4, p = 0.003, **Table 3, Figure 1a**). *Laboratory-reared* grasshoppers had higher survival rates than *field-caught* grasshoppers but the origin of the grasshoppers did not differ with respect to fecundity ($F_{1,6}$ = 1.2, p = 0.32, **Table3**, **Figure 1 b**).
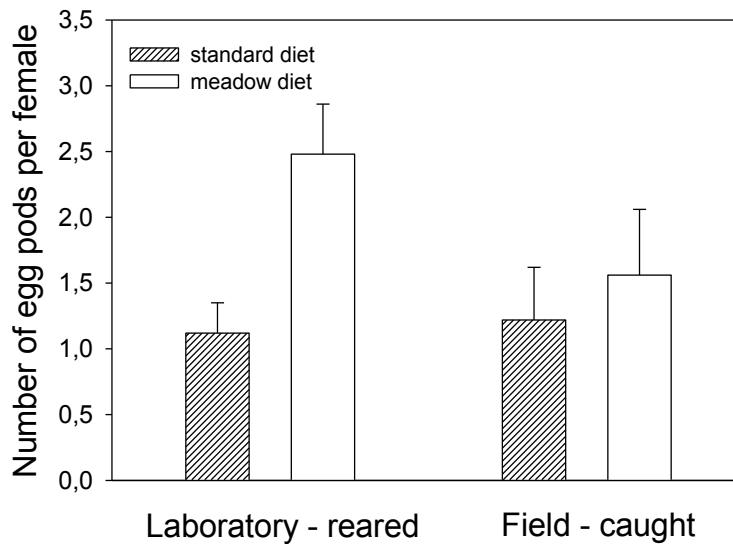
(a)
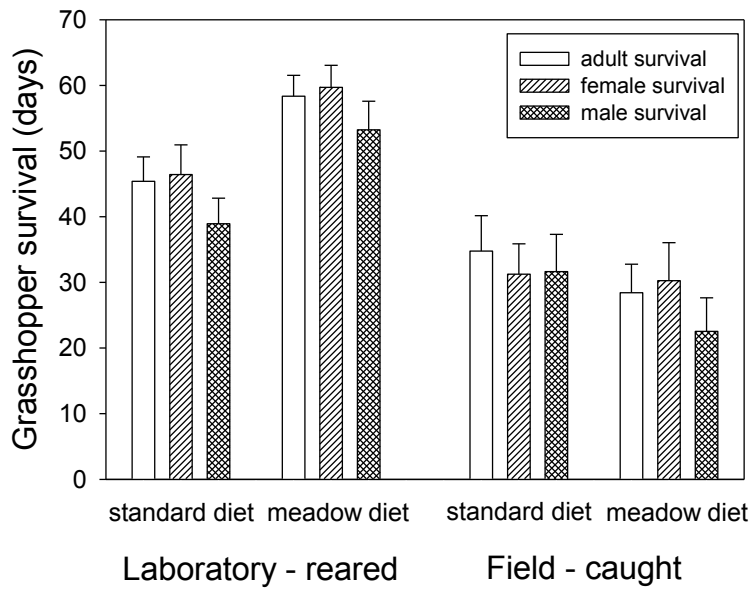
(b)

Figure 1 a-b: Performance of (a*) laboratory – reared* grasshoppers (left) and *field – caught* grasshoppers (right) in different diet treatments (*meadow diet* vs. *standard diet*) (white bar = adult survival, left striped bar = female survival, squared bar = male survival). Fecundity of (b) *laboratory – reared* grasshoppers (left) and *field – caught* grasshoppers (right) in different diet treatments (left striped bar = *standard diet*, white bar = *meadow diet*). Bars represent means ± S.E.

Carbon-nitrogen ratios in diets, grasshoppers and faeces

The *standard diet* showed a significantly lower C/N ratio than the average *meadow diet* (t-test: N = 12, df = 11, p < 0.001, **Figure 2**). *Laboratory-reared* grasshoppers fed on the *standard diet* had overall lower C/N ratios than grasshoppers fed on *meadow diet* (female: $F_{1,8} = 28.3$, p < 0.001; male: $F_{1,8} = 28.3$, p < 0.001; **Table 3**). C/N ratios in *field-caught* grasshoppers did marginally not differ with respect to the two different diet types (females: $F_{1,5} = 4.8$, p = 0.07; males: $F_{1,6} = 4.52$, p = 0.07; **Table 3**). Furthermore, there were no significant differences in body C/N ratio among the different populations (*laboratory-reared*: females: $F_{8,8} = 1.4$, p = 0.3; males: $F_{8,8} = 0.79$, p = 0.62, *field-caught*: females: $F_{8,5} = 1.6$, p = 0.3; males: $F_{8,6} = 0.99$, p = 0.51).
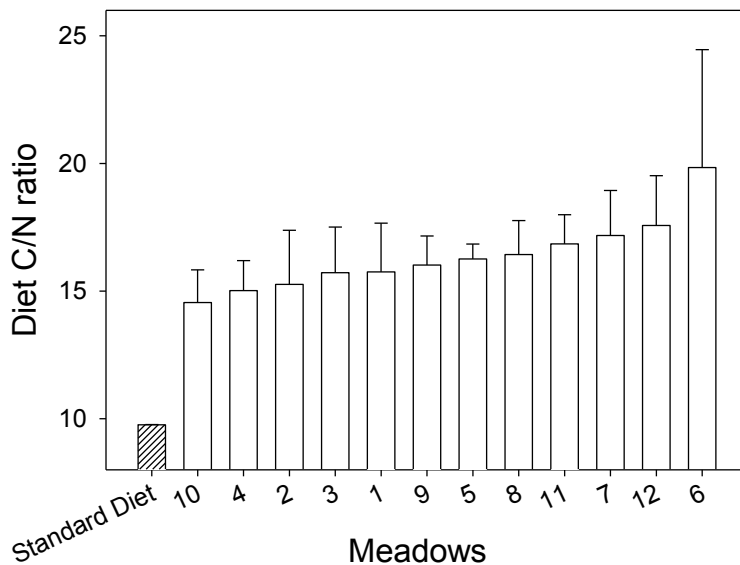

Figure 2: Diet C/N ratio in dependence of *standard diet* and the different *meadow diets* (left striped bar = *standard diet*, white bar = *meadow diet*). *Meadow diets* numbered accordingly the original meadows in the BIOLOG DIVA project (see supplementary material **Table S1**). Diet C/N ratio per *meadow diet* is calculated as mean ± S.E. out of single plant C/N ratios belonging to the specific *meadow diet* (for details see **Table 2**). *Standard diet* C/N ratio is calculated as mean ± S.E. out of single plant C/N ratios of *Dactlyis glomerata* and *Trifolium pratense* which served as food plants for the *standard diet*.

Grasshoppers that fed on a *standard diet* excreted more nitrogen than grasshoppers feeding on a *meadow diet* (*field- caught*: $F_{1,9} = 28.7$, p < 0.001; *laboratory- reared*: $F_{1,9} = 26.6$, p < 0.001, **Table 3, Figure 3**). The C/N contents in the faeces of *laboratory-reared* and *field-caught* grasshopper did not significantly differ between populations (*laboratory – reared:* $F_{9,9} = 0.8$, p = 0.6; *field – caught*: $F_{10,9} = 1.8$, p = 0.2). The measured C/N ratio which remained in the grasshopper bodies C/N turnover,

calculated out of the difference between C/N ratio diet minus the C/N ratio of grasshopper faeces, was much more negative in grasshoppers fed on *standard diet* than from grasshoppers fed on a *meadow diet* in both *origins,* but in *field- caught* grasshoppers marginal not significant (*laboratory-reared* grasshoppers: $F_{1,9}$ = 11.1, p = 0.009, *field-caught* grasshoppers: $F_{1,9}$ = 3.9, p = 0.07; **Table 3**). This implies that *standard* fed grasshoppers accumulated more nitrogen than those fed on the *meadow diets* (see **Figure 4**). Grasshopper *origin* did not significantly influence the C/N turnover (= difference between C/N plants and C/N faeces, ingested minus egested C/N) ($F_{1,8}$ = 0.18, p = 0.7, **Figure 4**).

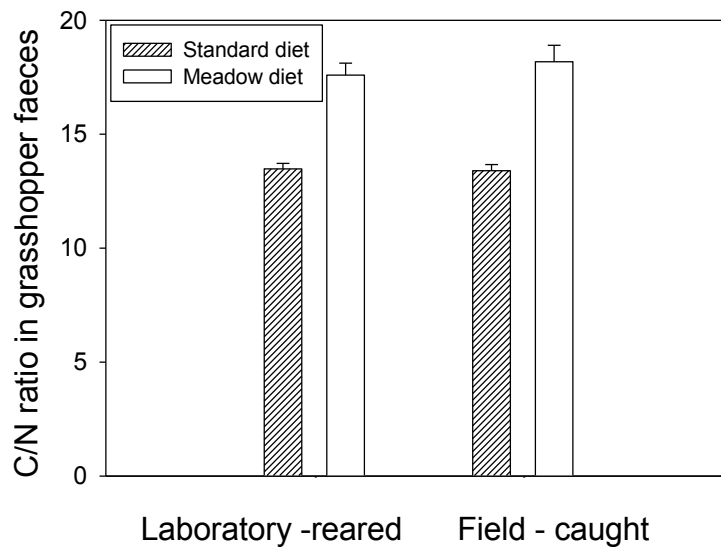
Figure 3: C/N ratio of faeces in *laboratory - reared* grasshoppers (left) and *field - caught* grasshoppers (right) in dependence of fed diet (left striped bars = *standard diet*, white bars = *meadow diet*). Bars represent means ± S.E.
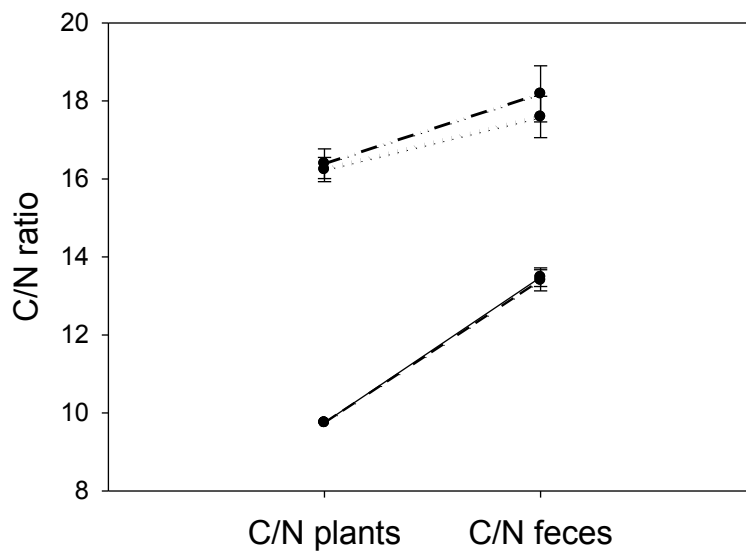
Figure 4: C/N ratio plants (potentially ingested) and C/N ratio faeces (egested) in *laboratory-reared* and *field-caught* grasshoppers in dependence of fed diet (dashed line = *field - caught* grasshoppers fed on *standard diet*, solid line = *laboratory - reared* grasshoppers fed on *standard diet*, dashed dotted line = *field-caught* grasshoppers fed on *meadow diet*, dotted line = *laboratory-reared* grasshoppers fed on *meadow diet*).

Discussion

The results from our study clearly showed that *laboratory-reared* and *field-caught* grasshoppers differed in how diet affects survival and fecundity. While *field-caught* grasshoppers were not affected by the two different diet types offered, *laboratory-reared* grasshoppers showed enhanced fitness when they were fed with species-rich *meadow diet* as compared to *standard diet* containing only *Dactylis glomerata* and *Trifolium pratense* throughout their lifetime. The prominent effect of species rich *meadow diet* on the fitness of *laboratory-reared* grasshoppers is another example for the beneficial effect of dietary mixing on generalist phytophagous insects (Bernays et al., 1997; Bernays & Bright, 1993; Bernays et al., 1994). The composition of host plants in the diet of *C. parallelus* individuals also changed throughout the ontogeny and with respect to grasshopper sex (Unsicker et al., 2008). In the present study, where only two food plant species were offered in the *standard diet*, the nutritional requirements of *C. parallelus* nymphs may not have been met to sustain optimal development. Mainly two hypotheses have been brought forward to explain the beneficial effects of dietary mixing on generalist insect herbivores, namely the "nutrient complementation hypothesis" and the "toxin dilution hypothesis". To reach an optimal nutrient intake and therefore a good performance the nutrient complementation hypothesis states that insect herbivores expand their diet

as it is assumed that a single food plant does not contain all the necessary nutrients (Pulliam 1975; Rapport 1980). Along this theory, compensatory feeding has been found in generalist insect herbivores (Berner et al., 2005; Lee et al., 2002; Takeuchi et al., 2005, Unsicker et al., 2008). The toxin dilution hypothesis assumes that plant toxins are diluted by mixing up single food plants whereas the negative effect caused by the toxin is decreased (Behmer et al., 2002; Freeland & Janzen, 1974; Marsh et al., 2006; Singer et al., 2002). The *laboratory-reared* grasshoppers in this study fed with *meadow diet* containing at least four plant species very likely performed better just because they had a higher chance of getting the optimal diet throughout their development. Whether these beneficial effects by dietary mixing are due to nutrient complementation or toxin dilution cannot be disentangled with our dataset.

In contrast to *laboratory-reared* grasshoppers, *field-caught* grasshopper performance was not affected by the type of diet offered in the last nymphal instar and as adults. Mortality in *field-caught* grasshoppers was higher than in *laboratory- reared C. parallelus* individuals. A simple explanation to these differences in survival may be that grasshoppers caught in the field with sweep nets were stressed and even harmed, which resulted in high levels of mortality in the lab. These effects on mortality had then to be considered independent of the diet offered to these grasshoppers. Furthermore, pathogen or parasite infestations that frequently occur in grasshopper populations under natural conditions (Ingrisch & Köhler, 1998) may have caused higher mortality in *field-caught* grasshoppers than in *laboratory-reared* individuals. The *standard diet* that was suboptimal for the fitness of *laboratory-reared* grasshoppers in our experiment had no detrimental effect on the *field-caught* individuals. Especially early instar feeding has repeatedly been shown to be important for later development and fitness (Barrett et al., 2009; Colasurdo et al., 2009; Metcalfe & Monaghan, 2001; Unsicker et al., 2008). With full access to all potential host plants in their habitat, *field-caught* grasshoppers were certainly able to feed on a broad diet throughout their early development before they came to the lab in their third and forth instar. A negative effect of the inferior *standard diet* on these grasshoppers may have not been visible because as late instar grasshoppers, they were no longer sensitive to suboptimal food. Franzke et al. (2010) could show in their study that especially during early ontogeny, the diet breadth of *C. parallelus* grasshoppers is broader (Franzke et al., 2010) than later on in the development. For caterpillars it was shown that the early stage of larval development is an extremely critical phase as early larvae are more restricted in feeding than later instars due to smaller heads and mouthparts (Reavey, 1993). Hochuli (2001) has shown, that early instar larvae also feed more frequently on high quality food than later instars. A well-balanced diet of early instar grasshoppers under natural field conditions might also explain why *field-caught*

grasshoppers showed the same levels of female fecundity as *laboratory-reared* individuals, no matter on which diet type they were kept in the lab.

It has to be noted, that the eleven different grasshopper populations observed in this study did not differ with respect to the measured fitness parameters, neither in *field-caught* nor in *laboratory-reared* individuals. This result is in accordance with the results from the study by Franzke et al (2010), in which the fitness response of one single *C. parallelus* population to different diets varying in plant species richness and composition was investigated. In this study the fitness response did not differ between grasshoppers fed with different diets but from data on food intake it was calculated that *C. parallelus* mixed food plants in different ways depending on the food plant identity and composition (Franzke et al., 2010). Irrespective of host plant identity, grasshoppers as plastic feeders may forage for plants that have chemical attributes which allow these generalist insects to reach their target intake and thus benefit their overall fitness.

The *standard diet* in our experiment was highly different from the *meadow diets* in terms of its C/N ratio. Against the well known "nitrogen limitation hypothesis" which states that presence and concentration of nitrogen in food plants is considered to be the most important factor affecting herbivore performance (Davison, 1995; Mattson, 1980; White, 1993) our study showed contrary results. In *laboratory-reared* grasshoppers the performance on the nitrogen-rich *standard diet* was significantly lower than on the *meadow diet* even though the C/N turnover data showed that more N was accumulated in grasshoppers that fed on the *standard diet*. These results emphasizes that nitrogen content alone is only a weak predictor of food quality and other plant nutrients, plant secondary metabolites and physical properties are at least as important for herbivore host plant quality (Awmack & Leather, 2002; Schoonhoven et al., 1998).

To conclude, experiences in early larval development of the generalist insect herbivore *C. parallelus* in this study revealed to be an important factor influencing performance responses when offered different diets later in life. For grasshoppers reared in the laboratory throughout their lifetime, a diet rich in host plant species was more important for the fitness than a nitrogen-rich but species-poor diet. In contrast, when grasshoppers spent their early instars in the field, there was no effect of diet on late instar and adult performance any more. The advantage of a broad and diverse diet for the grasshopper *C. parallelus* is also underlined by the positive correlation between fitness and plant species richness in the study area (Unsicker et al., 2010, Chapter I) and stresses the importance of diversity even for widespread and generalist herbivorous insects such as grasshopper *C. parallelus*.

Acknowledgements

Table S1: List of all grasshopper populations and corresponding population names according the names which were given to the meadows of origin in the BIOLOG DIVA project and the geographical coordinates of the meadows which are given in north/east. Information about the catching date of females for egg-laying of the *laboratory-reared* grasshopper treatment and dates when nymphs for *field -caught* treatment have been collected.

Population	Name	Coordinates	Dates of females caught for egg-laying of *laboratory - reared* grasshoppers	Date nymph collection for *field -caught* treatment
1	Schlegel	50°24'33.29"N, 11°37'36.57"E	between August - September 2006	-
2	Oßla	50°28'3.03"N, 11°29'42.87"E	between August - September 2006	beginning of July 2007
3	Tschirn - 11	50°23'5.07"N, 11°26'43.42"E	-	beginning of July 2007
4	Teuschnitz Aue -13	50°24'43.64"N, 11°23'15.41"E	between August - September 2006	beginning of July 2007
5	Steinbach	50°26'54.56"N, 11°24'23.85"E	between August - September 2006	beginning of July 2007
6	Tettauch	50°28'43.57"N, 11°15'45.06"E	between August - September 2006	beginning of July 2007
7	Tschirn - 20	50°24'31.43"N, 11°27'28.17"E	between August - September 2006	beginning of July 2007
8	Tschirn - 21	50°22'58.21"N, 11°26'34.27"E	-	beginning of July 2007
9	Tschirn - 22	50°22'53.95"N, 11°27'12.47"E	between August - September 2006	beginning of July 2007
10	Tschirn - 23	50°23'44.88"N, 11°27'24.49"E	between August - September 2006	beginning of July 2007
11	Teuschnitz Aue - 24	50°24'49.79"N, 11°22'48.80"E	between August - September 2006	beginning of July 2007
12	Saaleaue Jena	50°57'1.80"N, 11°37'14.03"E	between August - September 2006	beginning of July 2007

Table S2: List of C/N ratio of every food plant per diet, offered to the different grasshopper populations in the lab (g= grass, l = legume). Approximately five to seven individuals from each food plant species of each diet were dried in the oven for 48 hours on 70°C and ball milled. Shortly before they were C/N analyzed they have been dried in the oven for 24 hours on 70°C again to prevent water in the samples.

food plant	grass / legume	C/N ratio of plants in *meadow diet*												C/N ratio of plants in *standard diet*
		1	2	3	4	5	6	7	8	9	10	11	12	
Agrostis capillaris	g			16.98	16.99	16.11	12.76	19.9	11.2	-	20.5	18.73	17.18	
Anthoxanthum odoratum	g			24.67	18.31	16.81		20.39	15.54		17.52	19.93		
Arrhenaterum elatius	g					14.27			15.86	20.05	12.35	17.8	17.91	
Bromus hordeaceus	g												21.73	
Cynosorus cristatus	g			19.9						17.3				
Dactylis glomerata	g	21.12	16.04	13.18	13.25	17.7			23.36	-	18.05	13.06	14.12	9.77
Elytrigia repens	g		14.84										16.18	
Festuca rubra	g			11.61	13.52	15.5	31.93	22.02	22.59		15.53	22.6	28.11	
Holcus lanatus	g				18.85									
Holcus mollis	g			17.82			12.49	15.31				15.9		
Lolium multiflorum	g	20.51												
Lolium perenne	g	17.93							15.86	17.79				
Luzula campestris	g			22.07			22.18	20.47						
Phleum pratense	g	13.83	13.64			15.07			15.48	12.69	15.54	16.51	16.00	
Poa pratense	g	18.82	22.45			19.39					16.74			
Trisetum flavescens	g			15.19	13.77	15.28			13.72	13.91	12.08	15.68		
Lathyrus linifolius	l							11.24						
Trifolium pratense	l	10.33		8.09	10.48						9.49			9.75
Trifolium repens	l	8.17	9.36	7.75				10.91			7.69		9.31	
Vicia sepium	l											11.44		

Chapter IV

Taking Nutritional Ecology to the field: case studies with herbivorous insects

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Abstract

Generalist insect herbivores are able to enhance their fitness due to nutrient regulation. Under natural conditions nutrient regulation is a tool to respond to changing food plant qualities under an influence of specific abiotic effects. Both aspects seem to be close research fields in ecology. So far, the nutritional ecology mostly conducted experiments under artificial conditions that do not reflect natural scenarios, on the other side field ecology did not uncover mechanisms reflecting the relation between food plant quality and herbivore performance under field conditions, yet. The major goal of this review is to emphasize that the interplay between these topics would gain better knowledge about the fitness and performance of generalist insect herbivores under natural conditions. This is done by carving out the special position of *food plant quality* that causes under variation between food plant species the need of nutritional regulation. Also, the indirect influence of abiotic factors on herbivore performance is often documented due to a change in food plant quality. For that reason current knowledge on nutrient regulation in insect herbivores is shortly summarized and the experimental progress dealing with generalist insect herbivore performance by feeding on real food plants underlined by a biodiversity aspect is examined. Furthermore herbivore insect response due to the influence of abiotic factors mediated by changed food plant quality is described. Knowledge gaps were shown on all levels of research dealing with performance of insect herbivores due to either nutrient regulation or influencing abiotic factors. These gaps might be closed by a knowledge transfer between the different fields of research, which is underlined by our suggestions for experiments to combine these research areas at the end of this manuscript. The application of knowledge of nutrient regulation on natural based studies is the main appeal of this review.

Introduction

With a remark on the historical debate on what regulates communities in ecology (e.g. Andrewartha & Birch, 1960; Hairstone et al., 1960; Menge & Sutherland, 1987; Paine, 1966) plant resources, predators and abiotic conditions were specified as the three major factors influencing insect herbivore performance and abundance in the field (Chase, 1996; Kingsolver, 1989; Richards & Coley, 2008; Ritchie, 2000; Stamp & Bowers, 1990; 1994). The influence of abiotic conditions like temperature, solar radiation, moisture and CO_2 elevation on the abundance and performance of insect herbivores is due to the current climate change debate widely discussed in the literature (e.g. Cannon, 1998; Köhler et al., 1999; Laws & Belovsky, 2010; Stiling & Cornelissen, 2007; Unsicker et al., 2010). The limiting factor of predation and parasitism for insect population abundance and performance has also been pointed out (Branson, 2005; Lawton & Strong, 1981; Schmitz et al., 1997). Plants as food sources play an important role as supplier for water and nutrients but insect herbivores are often restricted by e.g. secondary metabolites and variable levels of nutrients in their food (e.g. Behmer, 2009; Bernays, 1998; Bernays et al., 1994; Mattson, 1980; Schoonhoven et al., 1998; Scriber & Slansky, 1981; White, 1984). Beyond that, the obtaining of protein is more limiting in herbivorous insects than in other major insect guilds because of the low levels of protein in plants (Mattson, 1980; Schroeder, 1986; Scriber & Slansky, 1981; White, 1978) which goes along with the nitrogen limitation hypothesis (White, 1984). To maintain performance insect herbivores developed certain strategies to overcome the disadvantage of unbalanced food in terms of nutrients like compensatory feeding and dietary mixing mixing (e.g. Bernays et al., 1992; Bernays et al., 1994; Hagele & Rowell-Rahier, 1999; Miura & Ohsaki, 2004; Unsicker et al., 2008; Behmer, 2009; Chambers et al., 1995; Howard et al., 1994; Raubenheimer & Simpson, 2003; Waldbauer et al., 1984). In this context, nutritional ecology research, dealing with the constraints of food in relation to insect herbivore feeding behavior and performance, experienced a rising interest in the past decades. A variety of work was published about the influence of food, nutrients and nutrient regulation on the behavior and performance on mostly generalist insect herbivores (Raubenheimer & Boggs, 2009), nevertheless relation to real and natural scenarios comprising for example abiotic influences were mostly neglected. However, to determine generalist insect herbivore performance in the field both factors, like plants as food and nutrient source and the effect of abiotic factors are important. Beside direct influences of abiotic effects the change of food plant diversity, composition and quality mediated by abiotic influences affects herbivore performance also indirectly in the field. Literature dealing with generalist insect herbivore performance under (semi-) natural conditions proved the positive effect of enhanced food plant diversity and quality (Unsicker et al., 2008; Unsicker et al., 2010) but pointed also out that insect herbivore performance in the field is not only determined by food plant resources alone (Franzke et al., 2010).

In this review we focus on generalist chewing insect herbivores which are known to switch food plants to be able to balance nutrients in their diet (dietary mixing) whereas this circumstance enables them to live in habitats differ in the composition and diversity of food plant resources. The latter aspect is very important due to a rising interest of plant species diversity effects on insect performance in field ecology. Therefore we define all poly- or oligophagous insect herbivores, feeding on more than one food plant species as generalists. The aim of this review is to combine the knowledge of nutritional and field ecology to understand how generalist insect herbivore performance is determined by food plant quality but furthermore also by composition and diversity which are all in turn influenced and affected by abiotic factors in the field. Therefore in this review, we do not cover the literature on the basics of nutritional ecology like the mechanisms of compensatory feeding and dietary mixing which is already nicely reviewed by others (Behmer, 2009). Abiotic effects play a significant role in this review but not as direct effects on herbivore performance but as mediators responsible for changed food plant quality and therefore changed nutrient regulation and performance in generalist insect herbivores under natural conditions. In this context, an important achievement of this review is to give an impulse to a new discussion how to merge the knowledge of field and nutritional ecology for a better understanding of how field studies can be supported by knowledge about herbivore performance in relation to nutrition regulation (see **Figure 1**).

1st section: Insights from the field of nutritional ecology

The question of the needs and constraints that insect herbivores face when they feed on plants stands in the center of the field of research dealing with insect herbivore nutrient requirements (Bernays, 1998) especially as the nutrient allocation in plants are not the ideal due to shortage of protein as only one example (Schoonhoven et al., 1998). The most important nutrients rather called macronutrients are nitrogen, carbohydrate and lipids for insect herbivore diet. Nitrogen (N) as an essential macronutrient is the main component of amino acids and proteins (and they are necessary for e.g. structural purposes, enzymes, storage & transport and for physiological functions). Although nitrogen is essential to herbivores the total nitrogen content in a plant is a poor index for its nutritional value (Schoonhoven et al., 1998). Carbohydrate (C) as provider for energy can also be synthesized from fats or amino acids, nevertheless they provide energy and their needed ratios can vary among herbivore species. Lipids which serve as storage for energy are fatty acids, phospholipids and sterols, whereas sterols are again essential for insects.
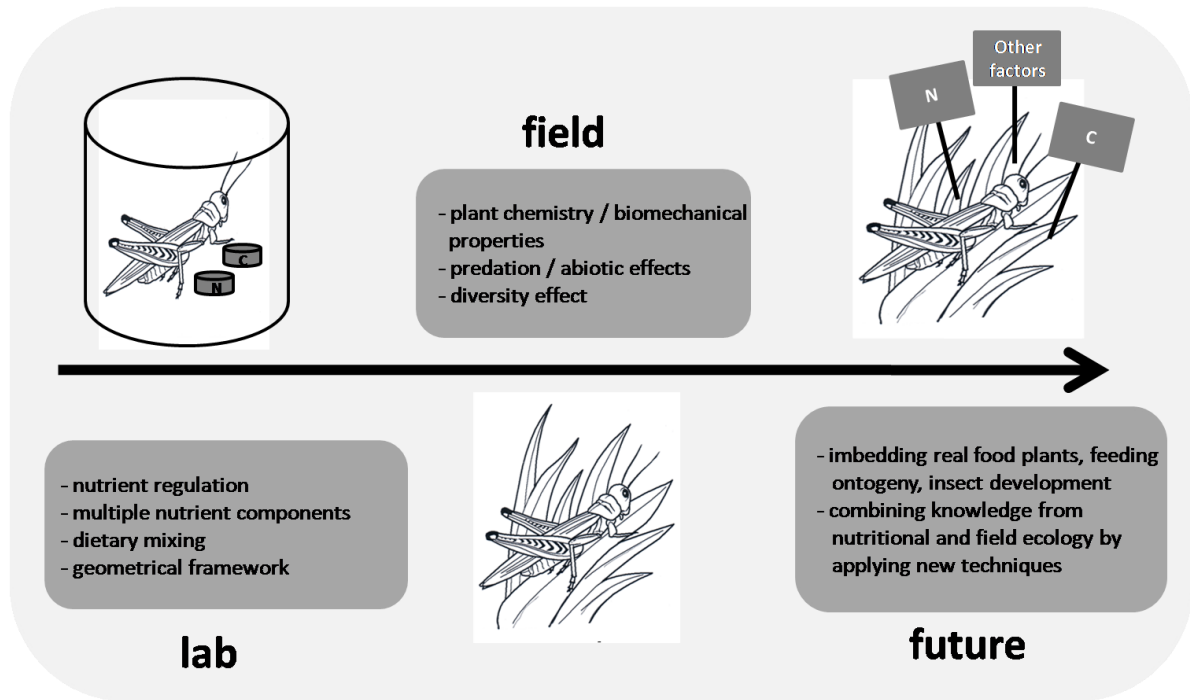
Figure 1: The timeline pictures the 3 chapters of this review. Firstly the achievements of the nutritional ecology are presented by pointing out that most studies have been done under laboratory conditions. In the field mostly correlative studies have been conducted yet, the figure highlights therefore the importance to go into the field by pointing out the influence of other factors which influences food plant quality directly and generalist insect herbivore response thereby indirectly. At the end the figure tries to picture what can and should be done in the future by combining the knowledge gained in the laboratory as well as in the field. (Source of grasshopper picture: www.jetztmalen.de)

To regulate nutrient intake insect herbivores evolved several mechanisms like pre- and postingestive mechanisms and learning (Behmer, 2009). Most realistic but also challenging is that food in terms of nutrients are often unbalanced therefore insect herbivores developed mechanism like compensatory feeding or dietary mixing to overcome this (reviewed in Behmer, 2009). Dietary mixing as one of the most prominent mechanism in nutritional ecology has been observed in several studies where herbivorous insects have either been fed with real food plants (e.g. Bernays et al., 1994; Hagele & Rowell-Rahier, 1999; Miura & Ohsaki, 2004; Unsicker et al., 2008) or artificial diets (e.g. Bernays et al., 1994; Chambers et al., 1995; Raubenheimer & Simpson, 2003; Waldbauer et al., 1984, reviewed in Behmer, 2009). As nature is not as simple insect herbivores are often faced with the complex situation of ingesting multiple food components at the same time, therefore Raubenheimer and Simpson (1993) developed the geometrical framework (GF) which addresses the ability of insects to ingest multiple nutrients in a changing and imbalanced nutritional environment (Behmer, 2009). However, food plants also contain nonnutritive secondary plant metabolites (allochemicals) which makes the situation worse for insect herbivores as to decide whether to regulate nutrient intake or ingest potentially toxic

components (Behmer, 2009; Bernays et al., 1994; Hagele & Rowell-Rahier, 1999; Singer et al., 2002) which goes along with the theories of nutrient balancing or toxin dilution (Freeland & Janzen, 1974). By applying the geometrical framework, experiments adding allochemicals to artificial food showed negative effects on insect performance in increased imbalanced diets in terms of the C/N ratio (Simpson &Raubenheimer, 2001).

The advantages of artificial food for nutritional ecologists are clear but also are the limitations, as feeding on natural plants is associated with several features of the food, physically like color, size, shape, toughness texture and chemically like odor and taste which is certainly missing in artificial food (Slansky & Rodrigues, 1987). However, green plants are far more than only carbohydrates and nitrogen and a high nitrogen level can be correlated to high concentrations of nitrogen containing secondary plant metabolites such as alkaloids that might reduce the digestive efficiency (Behmer, 2009; Simpson & Raubenheimer, 2001; Slansky & Rodrigues, 1987). Indeed, plants do have everything what herbivorous insect needs, like nitrogen, carbohydrates, lipids and water but they also come along with an unbalance of these macronutrients as well as secondary compounds like allelochemicals and toxins which are mostly anti-feeding stimulants for herbivorous insects. Furthermore, in laboratory studies proteins and carbohydrates are often seen as elements whereas this might work well for protein in the most cases. However, protein is seen as nitrogen (N) which is indeed a good predictor of protein (Behmer & Joern, 2012). In contrast, carbon alone is a bad predictor of total digestible carbohydrates in a plant (Behmer & Joern, 2012).

Although first attempts were made to test herbivore feeding behavior on real food plants with different biochemical pathways by mostly working on specialist insect herbivores (Behmer, 2009; Warbrick-Smith et al., 2009; Wright et al., 1999; Wright et al., 2003) applications on a real scenario between insect herbivores and several of their possible food plants are rare. In this regard most of the studies observed only insect growth, as only one fitness measurement over a short time period as reaction on nutritionally unbalanced food, although feeding ontogeny is known to change over several larvae stages and time periods (e.g. Dopman et al., 2002; Franzke et al., 2010; Sword & Dopman, 1999; Unsicker et al., 2008; Werner & Gilliam, 1984).

2nd section: The importance of going into the field

The next step after getting to know all the mechanisms of insect herbivore performance due to unbalanced artificial diets in the laboratory is to apply the knowledge into realistic scenarios. By adding factors like biotic and abiotic elements the disentangling of insect herbivore behavior and

performance response mediated by changed food plant quality are even more complicated but makes the situation more realistic in laboratory studies as well as field experiments. Environmental factors like predation, drought stress, elevated carbon dioxide and plant diversity therefore often indirectly influence foraging behavior and the performance of generalist insect herbivores. Furthermore, chemical and biomechanical properties of food plants often affect insect herbivore response in the field.

2.1 Chemical and biomechanical properties of food plants

The chemical nature of host plants as factor influencing feeding behavior of herbivorous insects has been in the focus of several studies with generalist insect herbivores in the past (Giertych et al., 2007; Harvey et al., 2005; Jansen & Stamp, 1997; Reudler et al., 2011; Stamp & Osier, 1997; Wright et al., 2003). It was shown that insect herbivores avoided feeding on leaves containing allelochemicals which underlines the theory of avoided adaptation to food plants due to containing allelochemicals (Wright et al., 2003). Beyond that, an increased developmental time due to feeding on allelochemical containing food plants was shown (Giertych et al., 2007; Harvey et al., 2005; Reudler et al., 2011). In addition it was shown that varying light and temperature regimes under natural conditions in the field interact with plant allelochemicals (either adverse additive or synergistic effective) leading to changed food plant quality and therefore influences herbivore performance (Jansen & Stamp, 1997; Stamp & Osier, 1997). The importance of biomechanical properties of food plant on insect herbivore performance is argued in several studies. The consequence of a reduced rate of nutrient supply due to increasing leaf toughness was shown by a poorer performance in a study using the locust *Chortiocetes terminifera* (Clissold et al., 2009). It was argued that rather access and not nutrient concentration *per se* may be the limiting factor (Clissold et al., 2009.)

2.2 Biotic and abiotic factors can change food plant quality and usage

2.2.1 Predation

Besides an increased mortality due to predation, the presence of predators can cause risk effects which are attended with changes in prey behavior under the presence of a predator. These nonlethal indirect effects (Dodson & Havel, 1988; Huang & Sih, 1990; Ludwig & Rowe, 1990; Skelly & Werner, 1990; Werner, 1991; cited in: Schmitz et al., 1997) are revealed due to an altered life – history schedule or habitat selection of the prey (Schmitz et al., 1997). Literature shows mostly a negative effect of predation pressure on herbivore behavior (e.g. foraging activities) (for more detail see **Table**

1). Danner and Joern (2003) pointed out that response on predation risk strongly depends on the developmental stage of the prey as the grasshopper nymphs of *Ageneotettix deorum* showed a reduced feeding activity and increased time spending in anti –predation behavior in the 3rd and 4th larvae stage but not significantly anymore in the 5th instar to adult stage on predation risk by a lycosid spider(Danner & Joern, 2003). Another study by this author underlined the perceived risk of spider predation on survival, growth and development rates of an insect herbivore in nymphs of *A. deorum* and additionally revealed the importance of high quality food and its potential to act in a compensatory manner for lost foraging time under predation risk (Danner & Joern, 2004).

2.2.2 Changing food plant quality due to drought stress

According to the plant stress hypothesis, foliar nitrogen is increased in physiologically stressed (e.g. by drought) plants (e.g. White, 1969; 1974; 1984) which makes the stressed plant due to the nitrogen limitation hypothesis to a favorable food plant. In contrast, the plant vigor hypothesis by Price (1991) states that many herbivore species feed preferentially on vigorous plants, opposite to the plant stress hypothesis (Price, 1991). The pulsed stress hypothesis (Huberty & Denno, 2004) was constructed on the discrepancy by observations in nature and controlled experiments regarding the plant stress hypothesis as it proposed a benefit for sap feeding insects through attacks of stress and the recovery of turgor which leads to a stress induced increase in plant nitrogen. Insect herbivore response on drought stressed food plant as an effect of increased temperatures showed very heterogeneous results, while plants reacted mostly as proposed with increased foliage nitrogen (see **Table 1**). However, Mopper et al. (1992) discussed heterogeneous findings of herbivore performance due to drought stress in the way that only long term sustained moderate plant stress affects the fitness as simultaneous plant stress while herbivores are feeding or oviposit can harm insect performance when examined on a small time scale (Mopper & Whitham, 1992).

2.2.3 Host plant phenology shift due to warming

Another influencing abiotic aspect on herbivore performance mediated by food plants is a shifted phenology of insect herbivores and its associated host plant species due to warming. This is mostly negative for the insect herbivore as it is shown in the prominent example of the generalist lepidoptera the winter moth *Operophtera brumata* (for details see **Table 1**).

2.2.4 Changing food plant quality due to elevated CO_2 concentrations

A rising interest within the climate change debate is the anthropogenic elevated carbon dioxide concentration which also impacts plant –herbivore interactions by its indirect effects on plant characteristics. Therefore elevated CO_2 concentration changes - beside plant physiology and morphology- also important host plant characteristics like plant chemistry and on that account food plant quality due to reduced or diluted nitrogen and increased phenolics (Buse et al., 1998; Hilbert et al., 1991; Lambers, 1993). The effect of e.g. increased tannin concentration in foliage reduces the abundance of many insect herbivores especially generalist feeders (Bernays & Chapmann, 1994; Bernays et al., 1989; Stiling & Cornelissen, 2007) but different herbivores may respond differently to changes due to elevated CO_2 (for details see **Table 1**).

2.3 Plant diversity effects on insect herbivore performance

As generalist insect herbivores occur in habitats differing in food plant species richness and composition they need to cope with different food plant species as well as with a varying number of them. Insect herbivore performance associating with plant species richness and diversity was often neglected in ecological research as well as in contribution to explain feeding ecology of insects in the field (Pfisterer et al., 2003). A few studies with the grasshopper species *Chorthippus parallelus* have been conducted on this field and they are drawing a confounding picture at the first sight. Beside the fact that dietary mixing also enhances performance in this grasshopper species (Unsicker et al., 2008) a significant positive influence of plant species richness in the field on grasshopper performance measured as fecundity was detected (Unsicker et al., 2010). In contrast, a cage experiment with *C. parallelus* found no relation between grasshopper performance and plant diversity (Specht et al., 2008), but an insurance effect of dietary mixing if the preferred food plant was not available was shown. Anyhow, the contrasting results suggested that plant species richness alone is not sufficient to explain the differences in grasshopper performance based on the possibility of mixing the best food

Table 1: The influence of biotic and abiotic factors on food plants and generalist insect herbivore performance. This table is presented as exemplary and represents only a choice of studies which have been conducted.

Biotic/ Abiotic Effect	Effect on food plant	Herbivore species	Direction of effect	Generalist insect herbivore response	Citation
Predation	no effect	several i.e. : *Melanoplus femurrubrum*, *Eritettix simplex* (grasshopper)	negative	change of foraging activities(lowering food intake, acceptance of lower food quality) in presence of predator	Lima 1990, Rothely 1997, Schmitz 1997, Danner 2003 a,b
		Melanoplus femurrubrum (grasshopper)	negative	shift in diet selection and shifted daily activity time under predation pressure	Schmitz 1997
		Schistocerca americana (grasshopper)	negative	dietary mixing influenced by food plant distance due to anti-predation behavior	Bernays 1997
Anti-predation behavior		*Grammia geneura*, *Estigmene acrea* (Lepidoptera)	negative	divided attention with less foraging efficiency of generalist insect herbivores due to anti-predation behavior	Bernays 2004
Drought stress	nitrogen accumulation due to drought stress, decreased vigor in drought stressed food plants	*Spodoptera litteralis* (Lepidoptera)	positive	higher leaf consumption and better growth on high drought stressed food plants	Mody 2009
		Chromatomyia milii (grass miner)	negative	feeding and oviposition preference positively related to water supply	Scheirs 2005
		Phoetaliotes nebrascencsi, *Ageneotettix deorum* (grasshopper)	neutral	no great effect in performance by plant stress due to drought	Joern 2005
Warming	host plant phenology shift due to warming	*Operophtera brumata* (winter moth)	negative	starving by hatching before oak bud burst, forced to feed on older leaves containing tannins by hatching after oak bud burst	Buse 1998, Feeny 1970, van Dongen 1997, Visser 2005
		Melanoplus sanguinipes (grasshopper)	positive	growth increase because of enhanced efficiency of using high plant quality resources because of asynchronous pulses of plant growth due to spatial variation in temperature	Searle 2010

Biotic/ Abiotic Effect	Effect on food plant	Herbivore species	Direction of effect	Generalist insect herbivore response	Citation
Elevated CO$_2$	changing plant physiology, morphology, chemistry due to reduced or diluted nitrogen and increased phenolics	*Operophtera brumata* (winter moth)	neutral	no affection by reduced leaf nitrogen in elevated CO$_2$ leaves	Buse 1998
		Antheraea polyphemus (Lepidoptera)	negative	slower developmental rate by feeding on oaks species grown under elevated CO$_2$	Knepp 2007
		Miramella alpina (grasshopper)	negative	changes in leaf water, nitrogen and starch concentration due to elevated CO$_2$ affected growth rates and egg mass in dependence of plant species and nymph developmental stage	Asshoff 2004
		Melanoplus femurrubrum (grasshopper)	positive, but mitigated due to plant species richness	higher growth rate under elevated CO2, but this was less pronounced for polyculture-than for monoculture grown food plants	Strengbom 2008

plant diet out of a potpourri of food plant species. Rather other conditions in the field like predation, abiotic influences and/ or the structure of the vegetation must lead to this result.

This explanation is supported by the study of Franzke et al. (2010) that showed homogeneity in performance by feeding on diets differing in plant species richness and composition, which was achieved through differences in the conversion efficiency of food to body mass. This study emphasized that, when given the choice, the grasshopper species *C. parallels* mixed the different diets in a way that results in a similar fitness so that it is not the plant quality per se which brings the differences in grasshopper performance among different habitats assumingly a quorum of good quality food is available.

It was shown, that feeding behavior and insect herbivore performance is changing due to chemical and biomechanical properties in the food plants, by predation and fluctuating abiotic factors (e.g. increased temperature, elevated CO$_2$) and that this is mediated by changed food plant chemistry and quality. However, the described experiments and observations, which have mostly been correlative studies, revealed a heterogeneous response of insect herbivores on changing food plant quality. The truth however, lies possibly among several factors for example in the single insect life history (insect ontogeny) or in the observed insect species (insect guilds react differently) or even in the specific ability of each insect herbivore species to compensate for a changed food plant quality. To underline the latter point it might be very useful to extent the observation of insect performance on abiotic factors by including knowledge gained from studies in the field of nutritional ecology.

While trying to figure out how the performance of herbivorous insects is determined in the field based on the assumption that food has one of the most important influence we cannot avoid to become more complex by testing hypothesis of the nutritional ecology under field conditions or at least with real food plants. The results have been documented in detail above but the knowledge of how insects regulate nutrients under field conditions or by foraging on real food plants is still lacking.

3rd section: The missing link between nutritional ecology and field ecology– where do we want to go?

In the previous chapters, we showed that there is a need to combine the knowledge of field and nutritional ecology to gain a better understanding of insect herbivore performance by feeding on real food plants or under more natural scenarios.

Studies on the mechanisms of nutrient regulation proved with real food plants have been done in the past but the ontogeny of feeding as well as the extremely importance of insect development was mostly neglected. The missing links between laboratory nutritional ecology and the correlative studies done in the field will be subjected in the following section.

3.1 Mechanisms of nutrient regulation proved with real food plants

Studies related to nutritional ecology dealing with herbivorous insects foraging on real food plants confirmed theories of dietary mixing although their currency were, in contrast to studies conducted within the field of nutritional ecology, mostly not the observation of nutrients *per se* but furthermore the behavior and performance response by feeding on real food plants (Bernays et al., 1992; Bernays et al., 1994; Hagele & Rowell-Rahier, 1999; Miura & Ohsaki, 2004; Unsicker et al., 2008) (for more detail see **Table 2**).

3.2 *The influence of insect development &feeding ontogeny*

Most studies considering the influence of food plants and nutrients on feeding behavior and herbivore performance are mainly concentrating on adults and contain therefore a very restricted picture in the insect life. In fact, food plant quality has an overwhelming effect on the development of insect herbivores and it is therefore of extreme importance to observe also the ontogeny of feeding by studying the influence of food on herbivore performance in different stages of life.

Table 2: Insect herbivore response due to the mechanism of dietary mixing. This table is presented as exemplary and represents only a choice of studies which have been conducted

Tested mechanism	Herbivore species	Insect herbivore response	Citation
Dietary mixing (nutrient complementation and toxin dilution)	*Schistocerca americana* (grasshopper)	enhanced growth rate on mixed food plants than on single food plants	Bernays 1994
	Miramella alpina(grasshopper), *Callimorpha dominula* (lepidotpera), *Cylindrotoma distinctissima*(diptera)	enhanced growth rate on mixed food plants than on single food plants, mechanisms of either nutritional balancing or toxin dilution remained unclear	Hagele 1999
	Parapodisma subastris (grasshopper)	greatly improved survival rates by feeding on mixtures of food plants even of individually worse food plants	Miura &Ohsaki 2004
	Chorthippus parallelus (grasshopper)	showed a higher fitness (survival, fecundity) by feeding on a mixed rather than a single diet	Unsicker 2008
	Orygial eucostigma S. (lepidoptera)	foligae mix of different age classes leads to enhanced fitness	Johns 2009
	Lymantria monacha L. (lepidopera)	no effect in a mixed forest type (spruce and beech) in comparison to single forest type (either spruce or beech), abundance decrease with rising beech ratio	Heiermann 2008
	Parapodisma subastris (grasshopper)	mass gain of nymphs fed on a mixture of two inferior plants was greater than feeding them singly, also mass gain did not differ by feeding on one single superior plant	Miura &Ohsaki 2006
	Grammia geneura(Strecker) (lepidopera)	first experimental evidence for toxin dilution process and for combined influence of nutrients and secondary metabolites of caterpillars foraging pattern in nature	Singer 2002

In this respect, the grasshopper species *Chorthippus parallelus* showed a change in food preference depending on the developmental stage and sex (Franzke et al., 2010; Unsicker et al., 2008). A significant different response on a broad vs. a narrow diet was the result of a study with the same grasshopper species (see Chapter III) whereas one grasshopper treatment were fed with suboptimal food over the whole life and grasshoppers from another treatment were in turn fed with this suboptimal diet starting in a later point of life. Hence, late instar grasshoppers were not very sensitive to suboptimal food due to an optimal supply of resources during early instars which might act as an insurance for bad times in later life. However, feeding experiences in early larval development

revealed to be an important factor influencing performance responses when offered suboptimal diet later in life (Chapter III). This assumption is underlined by the study where poor quality diet in a juvenile stage leads to a prolonged juvenile development and therefore a shorter lifespan in the cockroach *Nauphoeta cinerea* (Barret et al., 2009). Other studies confirmed that a poor start in life due to e.g. a shortage of nutrients during early development can lead to enormous consequences on the adult performance even if the supply of resources improves during the development (Metcalfe & Monaghan, 2001). Changing climatic factors like periods of drought, prolonged rainfall influencing food plant quality or even a derived asynchronism of host plant and insect development are therefore potential factors accelerates a poor start in life under natural conditions.

3.3 Finally – combining knowledge from nutritional and field ecology

The knowledge about nutritional balancing gained in the laboratory regarding unbalanced nutrients, multiple food components and the combination with secondary compounds is still not applied in field ecology. In the field mostly correlative studies proved mechanisms like dietary mixing and highlights the importance of plant characteristics, predation and other factors influencing and changing food plant response and quality. Field ecology is often explaining results using background knowledge gained in the laboratory although this remains often speculative because methods of nutritional ecology have rarely been applied in field ecology so far.

At this point we would like to make some suggestions how to develop experiments combining field - and nutritional ecology. Therefore the following section in the form of a cooking recipe is addressed towards young scientists and can be seen as a suggestion to disentangle the questions of what should be known to gain knowledge about how generalist insect herbivores are foraging under field conditions. At first we think it is of great importance to gain basic nutritional knowledge of the single players like the herbivore and its associated food plants (see: Getting to know the 'players'). In a second step we propose to combine the gained knowledge within certain experimental approaches (see: What can be done to fill the knowledge gaps). At the end of this process the way is paved for studies and applications on different topics and questions dealing with the insect – food plant model system (see: 'To infinity and beyond'- The application on different topics and questions).

 (1) Getting to know the 'players'

The first step might be for most generalist insect herbivore species that used as model organisms a step back into the laboratory. The aim is to create realistic conditions in the laboratory to be able to observe the exact amount of food ingested by the herbivores. Thus, it is of great importance to

characterize the food plants in terms of nutrient amount and the amount of secondary metabolites to be aware of what the herbivore is foraging on. To disentangle the complex relationship of nutrient balancing which is in nature combined with e.g. plant chemistry, effect of other factors and predation it is of urgent importance to be really sure about how the study organisms both insects as well as plants are working. In this context, a useful workflow might be:

(a) Learning to know the intake target of the insect species.

(b) How is insect body composition changing over time?

(c) Do sexual dimorphism and size phenomenon plays an important role?

(d) Are early feeding experiences and feeding ontogeny influencing insect fitness over time?

(e) Do we argue on the population, community or another specific level?

(f) How is the degree of specialization /generalization in the herbivore?

(g) Characterize the nutritional environment and the nutritional value of the food.

(h) Quantifying the amount of digestible carbohydrates in a plant.

(2) What can be done to fill the knowledge gaps

Starting to work with real plants either in the field or under semi-natural conditions for example in the greenhouse and as well as in the laboratory is a clear must be by capturing nutritional ecology in the real world as this is not possible with artificial diets. As methods and mechanisms of nutritional ecology often bases on definite amounts of ingested food it is still extremely difficult to conduct field studies as a 24 hour observation of feeding over whole developmental periods is not really possible. A list of first steps by starting to combine nutritional and field ecology within an experimental approach could look like this:

(a) Repeat studies and observations which have been conducted with artificial food.

(b) Look beyond the nutritional requirements of protein and digestible carbohydrates by also embedding micronutrients like P (Behmer & Joern, 2012).

(c) Combine the knowledge of point 1 (Getting to know the players): Conduct correlative studies between plant nutrient content and herbivore performance in the laboratory as well as in the field, adding other factors (biotic & abiotic), compare studies in the laboratory and the field.

(d) To disentangle the ingested amount of food plant tissue, apply technologies based on molecular analysis of e.g. plant tissues in the insect gut.

(3) 'To infinity and beyond'- The application on different topics and questions

After gaining basic nutritional knowledge of the single players (see point 1) and the combination of both (see point 2) under certain laboratory or field environments the way is paved for ecological studies which (i) goes further and becoming more complex or (ii) where the nutritional behavior and response need not necessarily playing the first role but rather provides extremely explanatory power disentangling ecological relations concerning insect – plant relationships in a broader sense. In this context nutritional ecology could become more ecological as well as serve as a good tool dealing with following ecological questions:

(1) Which role is playing belowground ecology by observing insect-plant relationships?
(2) How is climate change influencing herbivore response by changing food plant quality?
(3) How respond insect communities in comparison to insect populations on changing food plant diversity and quality?
(4) How is the influence of functional diversity on insect performance?
(5) Which role is playing nutritional ecology in mass outbreaks of insects?
(6) …to infinity and beyond!

Conclusion

Expanding the knowledge of what determine generalist insect herbivore fitness mediated by food plant quality, diversity and composition was the intention of this review. Plant resources, predators and abiotic conditions are known to be influencing insect herbivores, however, in this review we proclaim that only the combination (interaction) of these extrinsic factors gives a holistic picture and leads to a better answer of the question what influences insect herbivore performance. Hence, by carving out the special position of food plant quality which is in turn mediated by habitat diversity, food plant composition and certain abiotic influences we emphasize the importance to combine these factors rather than to look at them singly while trying to understand herbivore performance. As novelty we additionally considered the self-regulatory aspect of insect herbivores that is expressed through the ability of outbalancing unfavorable nutrient concentrations in the food.

A combination of all of these factors influencing insect herbivore performance is not trivial at all, as this means to merge the knowledge and methodology of different ecological research fields which in turn might lead to complex studies and a more complicated general view.

Nutritional ecology as a major field of research in itself gained a great progress in the last decade and made therefore great contributions explaining intrinsic mechanisms of nutrition balancing under unfavorable conditions. However, the greatest point of criticism might here be the lack of application

under natural conditions. Most of the studies determining individual insect herbivore response to the challenge of feeding per se took place under ideal situations with artificial food, in laboratory studies and therefore strictly controlled conditions. To describe the fundamental mechanisms of ingesting multiple nutrients and allelochemicals on herbivore performance controlled studies are very important. However, it is of great importance to apply the derived knowledge to understand how insect herbivores are influenced in their fitness by feeding and living as normal as possible. Starting with real food plants is the first step and was already realized in very few studies (Warbrick-Smith et al., 2009), as feeding on real plants comprises more than only balancing nutrients and allelochemicals. As mainly generalist insect herbivores live in habitats varying in food plant species richness and composition it was several times proved that dietary mixing and compensatory feeding is here also the strategy to sustain and enhance herbivore fitness (Bernays et al., 1992; Bernays et al., 1994; Hagele & Rowell-Rahier, 1999; Miura & Ohsaki, 2004; Unsicker et al., 2008). Nevertheless complex studies with real food plants in the laboratory or field studies are often confounding and blurred due to different uncontrolled factors (Franzke et al., 2010; Specht et al., 2008; Unsicker et al., 2010, Chapter II & III). However, it would be of great advantage to test the ability of generalist insects to maintain fitness due to dietary mixing and complementary feeding with well-directed applications of how insects regulate nutrients under field conditions. Insect herbivore fitness is often indirectly influenced by factors of climatic or anthropogenic nature and this in turn is mediated by changed food plant quality but studies describing beside herbivore fitness also nutrient regulation are still lacking on this field of research. Hence, important information about the potency and ability of herbivores responding to changed food plant quality is missing and beyond that the forecast what might happen with herbivores under the influence of increasing climate change is not possible, yet.

Beside this, also the development of insects and therefore changed nutrient requirements during insect ontogeny should not be observed apart from each other rather than treated as a unit. Derived knowledge of herbivore response to either nutrient regulation on artificial food or herbivore fitness due to changed food plant quality mediated by biotic (food plant diversity, composition, chemical and biochemical properties of food plants, predation) or abiotic (temperature, elevated CO_2) factors should always be the mean response of a whole insect life rather than a snap-shot of a single life period.

Determining factors influencing herbivore performance is getting harder by becoming more complex and therefore real, the combination of factors like plant resources and abiotic conditions is only one step. To consider also the self-regulatory abilities of insect herbivores would give a really new insight into what determines insect herbivore performance in the field and bring a bit more ecology into the field of Nutritional Ecology.

Acknowledgements

General Discussion

The overarching question of this thesis was to understand the effect of diet, food plant species richness, composition and quality on the performance of the generalist insect herbivore *C. parallelus*. Hereby, single experiments, ranging from laboratory to natural field conditions, deliberately concentrated on different aspects of the focal question like the effect of plant species richness of different meadows, or the importance of early feeding experiences , or the possibility of local adaptation, or the influence of maternal effects**.**

Grasshopper fecundity was shown to be positively affected by plant species richness in a field survey. Furthermore the abundance of *C. parallelus* was positively correlated with plant species richness, plant community composition and solar radiation of the different meadows in the study area (Chapter I).

The hypothesis of *C. parallelus* being locally adapted due to differences in plant diversity between the meadows in the study area was not confirmed. However, a strong fitness advantage in grasshopper populations who´s parents had been reared on meadows of good conditions in terms of high plant species richness and a low biomass was revealed and is therefore a strong hint for active maternal effects, beside the fact that *C. parallelus* is known to be a plastic feeder(Franzke et al., 2010)(Chapter II).

In this study it could be shown (Chapter III) that grasshopper *origin* strongly effected susceptibility to diet effects in *C. parallelus*, as populations reared in the laboratory showed a higher performance on the broad *meadow diet*, while in *field-caught* grasshoppers broad or narrow diet did not influence performance.

The influence of early larval (feeding) experiences on grasshopper performance

The influence of early larval (feeding) experiences acting on grasshopper performance is an important finding in this thesis. The experimental study presented in Chapter III showed different fitness response of *laboratory- reared* in comparison to *field-caught* grasshopper populations, whereas these results has been discussed within the framework of early larval (feeding) experiences. Although studies about the influence of feeding ontogeny are relatively rare, there is some evidence that emphasizes the importance of early instar feeding for later development and fitness in insect herbivores (Barrett et al., 2009; Colasurdo et al., 2009; Metcalfe & Monaghan, 2001; Unsicker et al.,

2008).A study with the cockroach *Nauphoeta cinerea* for example shows that good juvenile conditions in terms of a high-quality diet resulted in faster juvenile growth and longer reproductive lifespan (Barrett et al., 2009). A laboratory feeding experiment where one population of the study organism *C. parallelus* were fed with five different meadow diets, showed that especially during early ontogeny, the diet breadth of the grasshoppers is broader than later on in the development (Franzke et al., 2010). It was furthermore shown that early caterpillar instars comprise an extremely critical phase as early larvae are more restricted in feeding than late instars due to smaller heads and mouthparts (Reavey, 1993). Hochuli (2001) has shown that early instar larvae also feed more frequently on high quality food than later instars. Thus, good juvenile conditions in terms of a well-balanced diet promises a better adult performance but might also serve as an insurance for later life. Therefore the non-reaction on the two different diets (*meadow vs. standard*) of *field- caught* grasshoppers (Chapter III) could be due to the fact that they were able to feed on a broad diet through their early development in the meadow habitat before they had been transferred into the laboratory.

Also differences between studies on the performance of *C. parallelus* in response to plant species richness might be due to early larval (feeding) experiences and are discussed at this point. Within this context, the positive relationship of plant species diversity on grasshopper performance presented in Chapter I (Unsicker et al., 2010) and shown by measuring fecundity of females which had been caught on different meadows was not revealed in grasshopper performance and fecundity along a gradient of plant species richness in a field biodiversity experiment (Specht et al., 2008). However, these results are based on studies with substantially different approaches: In the correlative study of Chapter I grasshoppers grown on real meadows differing in plant species richness and composition had been caught in the adult stage to measure fecundity. In contrast, the cage experiment by Specht et al. (2008) caged field-caught grasshoppers of *C. parallelus* in their 4th nymphal stage on plots along a gradient of plant species richness within a biodiversity experiment. It is therefore assumed, that possible early feeding experiences during grasshopper development over the first four nymphal stages might have blurred the effect of plant species richness on grasshopper performance in the second approach.

The influence of maternal effects on grasshopper performance

The impact of maternal effects influencing relocated grasshopper population performance has been discussed due to the results shown in Chapter II. In this context a high parental fitness as well as good conditions in the parents habitat (e.g. high plant species richness, low biomass) leads to a fitness advantage in relocated offspring which were particular evident in cases where the *experimental*

meadow showed a difference in the *meadow attributes* (plant species richness, biomass) in comparison to the *meadow of origin*. In this context, it is of no surprise that *C. parallelus* populations has been proven to be not genetically different between the meadow habitats in the study area (Wiesner et al., 2011) as maternal effects do not results in a genetic change of the offspring. However, maternal effects are rather described as the influence of parental environment which leads to the transfer of information from maternal generation to the phenotype of the offspring (Hunter, 2002; Mousseau& Fox, 1998). Nevertheless, literature states that various attributes can serve as parental environment like climate, food quality, food diversity and predation pressure (Hunter 2002) and in fact studies dealing with the influence of food plant quality (e.g. allelochemistry of parental diet, lack of essential nutrients in parental diet) shown to be influence offspring performance (Behmer & Grebenok, 1998; Gould, 1988; Hunter, 2002) and these effects were referred to the influence of maternal effects. By examining population dynamics of gypsy moth in the field that are discussed to be driven by maternal effects it was pointed out that a clear evidence in the field is still missing (Hunter, 2002). The great problem in this context is to disentangle the influence of maternal effects from other effects operating in the field (Hunter, 2002). The possibility of maternal effects influencing grasshopper performance of *C. parallelus* are evident and expressed in the study (Chapter II) but more detailed investigations are needed to exclude other effects effective in the field.

Nevertheless, it is of importance to underline that from the measured environmental factors influencing parental fitness and therefore offspring fitness due to maternal effects, plant species richness was shown to be of outstanding relevance. These findings (Chapter II) underlines the positive effect of increasing plant species richness on grasshopper performance already shown in the observational study presented in Chapter I. The mechanism which is working by discovering a positive relationship between increasing plant diversity and insect herbivore performance is discussed in detail below.

Dietary mixing hypothesis – a predictor for generalist insect herbivore performance in habitats diverse in food plant species!

Apart from the exclamation mark at the end of this subheading, the dietary mixing hypothesis is introduced under the same heading in the general introduction and was furthermore discussed in every chapter of this thesis, which is underlining the outstanding importance of this hypothesis in the present thesis. Under the assumption that macronutrients are unevenly distributed among food plants it is beneficial for generalist insect herbivores to mix a diet consisting of different food plant species to complement their nutritional status (nutrient complementation hypothesis)(Pulliam, 1975; Rapport,

1980).Grasses, being the main food plants of the model organism *C. parallelus,* have been shown to have relatively few intrinsic toxins (Cheeke, 1995). Therefore it might be assumed that the toxin dilution hypothesis is irrelevant for this herbivorous grasshopper species. On the contrary, there are also studies showing that grasses might contain fungal metabolites, so called mycotoxins, which contribute to the grass defense against herbivory (Cheeke, 1995) and might call for the toxin dilution hypothesis(Behmer et al., 2002; Freeland & Janzen, 1974; Marsh et al., 2006; Singer et al., 2002).However, the experiments of this thesis never examined the fungal infestation status of the food plants nor other secondary metabolites. In these as in most other studies it is therefore hard to distinguish between the mechanism of 'nutritional complementation' or 'toxin dilution' within the dietary mixing hypothesis. The relative importance of both possibilities was suggested by Pennings (1993) and Bernays et al. (1994). The notice that mixtures of food items mostly improve the performance in comparison to single food was studied over the whole animal kingdom from sea hares (Pennings et al., 1993) over birds (Leeson & L.J., 1991), land slugs (Speiser & Rowell -Rahier, 1991), lizards (Dearing & Schall, 1992) mammals (Belovsky, 1984; Chung & Baker, 1991) to herbivorous insects like butterflies (Merz, 1959) and grasshoppers (Bernays et al., 1994; Hagele & Rowell-Rahier, 1999). Also the study organism *C. parallelus* showed a higher fitness by feeding on a mixed rather than a single diet (Unsicker et al., 2008) and therefore underlines the hypothesis of dietary mixing. In this context it is most likely to assume that enhanced grasshopper performance with increasing plant species richness is due to the possibility of dietary mixing, as with increased plant species diversity the amount of food plants also increases (Chapter I). Although three possibilities for the observed positive aspect of plant species richness have been described in Chapter I the ability for a better diet mix was proposed to be the most evident one. The fitness advantage of grasshopper offspring from parents originating from meadows with a high plant diversity might also be caused by the mechanism of dietary mixing in the parental generation. The fitness advantage of these parents was then transferred by maternal effects to the offspring generation (see Chapter II). The positive response in *laboratory – reared* grasshoppers feeding their whole life on the broad *meadow diet* gives a real evidence for dietary mixing in this generalist insect herbivore, although the two- plant species *standard diet* offered a higher proportion of nitrogen (see Chapter III). In this context it has to be emphasized that nitrogen alone is a weak predictor for food plant quality. This is arguing against the 'nitrogen limitation hypothesis' which describes nitrogen as the most important macronutrient of food plants and is affecting insect herbivore performance (Davison, 1995; Mattson, 1980; White, 1993). This hypothesis was discussed contradictory in the literature with studies underlining the hypothesis (Davison, 1995; Heidorn & Joern, 1987; Joern & Behmer, 1997; Ritchie, 2000) and others not supporting it (Cease et al., 2012; Fischer & Fiedler, 2000; Joern & Behmer, 1998; Ritchie, 2000, Chapter III).

Altogether, this thesis could clearly show that the dietary mixing hypothesis is indeed a good predictor for generalist insect herbivore responses on plant diversity. Nevertheless, within the discussions of the single Chapters belonging to this thesis it was stated that plant species diversity *per se* as a predictor for a positive grasshopper performance might not be the only factor influencing herbivore performance along a gradient of plant species richness and composition.

Insect herbivore performance in the field – the influence of biotic and abiotic factors

Living and foraging in the field challenges insect herbivores to a great amount as maintaining a good performance is in this circumstance depending on more factors than simply dietary mixing and nutrient regulation by foraging in grassland habitats (see **Figure 2**).

As one additionally factor in this context I would like to highlight the importance of plant community composition and plant community structure as surrogates for diverse effects on generalist insect herbivores. Plant community structure is shaped by the effects of plant species richness, functional richness and composition (see **Figure 2**) where plant community composition was shown to be positively affecting grasshopper abundance of *C. parallelus* in the field (results Chapter I). To disentangle the separate effects of plant composition and plant community structure (vegetation structure) is not easy as it combines several effects like good and bad food plants, microclimatic and structural effects such as refuges for anti-predation behavior (Perner et al., 2005). Especially for *C. parallelus* as a temperature depended grasshopper species microclimate is playing an important role for occurrence (Ingrisch & Köhler, 1998).

Chapter IV reviewed the influences of biotic and abiotic factors as direct and more specifically as indirect effects mediated by changed food plant quality on generalist insect herbivore performance in the field (see **Figure 2**). Hence, changed foraging activities of insect herbivores due to anti-predation behavior (e.g. Bernays et al., 1997; Bernays & Bright, 1993; Danner & Joern, 2003; Lima & Dill, 1990; Schmitz et al., 1997) as well as the influence of changed food plant quality on herbivore behavior and performance due to drought stress (e.g. Buse et al., 1998; Joern & Mole, 2005; Mody et al., 2007; Scheirs & De Bruyn, 2005) and elevated CO_2 (Asshoff & Hattenschwiler, 2005; Buse et al., 1998; Knepp et al., 2007; Strengbom et al., 2008) have been discussed. Furthermore the effects of plant species diversity in relation to the dietary mixing hypothesis (Bernays et al., 1992; Kaufmann, 1965; MacFarlane & Thorsteinson, 1980; Pulliam, 1975) on the foraging behavior and performance of *C. parallelus* (Unsicker et al., 2008; Franzke et al., 2010, Chapter I, Chapter II, Chapter III) and the often

neglected influence of insect development and feeding ontogeny (e.g. Barrett et al., 2009; Colasurdo et al., 2009; Metcalfe & Monaghan, 2001; Unsicker et al., 2008; Chapter III)were highlighted. As a result, different responses in herbivore performance to food plant diversity, quality and composition from studies conducted in the field or the laboratory should be interpreted with caution.
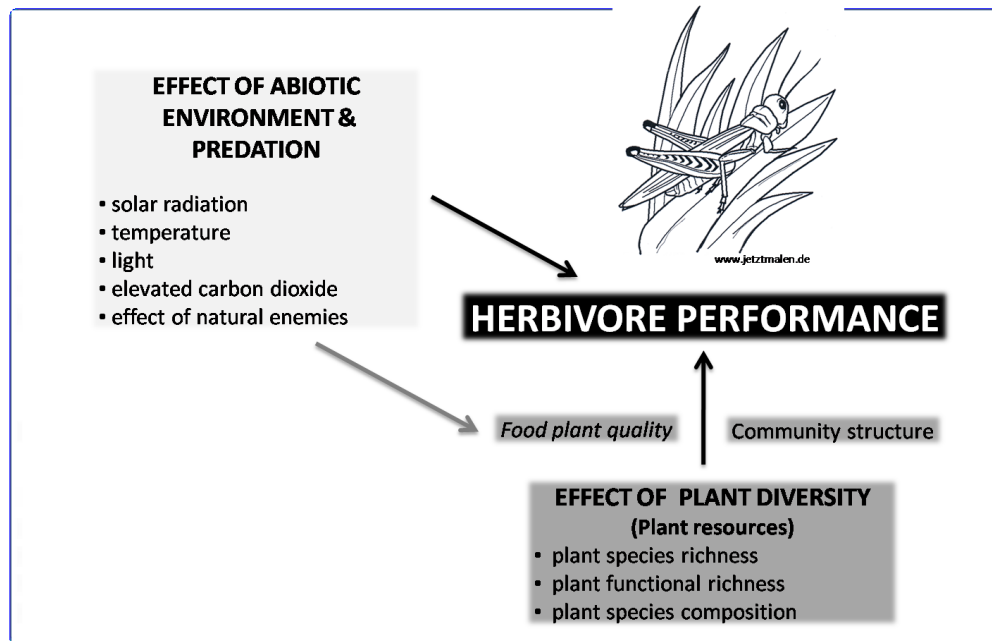

Figure 2: The relationship between plant resources (dark grey box) and abiotic factors & predation (light grey box) on generalist herbivore performance. The indirect influence of abiotic factors on herbivore performance which is mediated through changed food plant quality is expressed through the light grey arrow. The direct effects are displayed by black arrows.(Source of grasshopper picture: www.jetztmalen.de)

Grasshopper performance response on diet – are these studies contradictory?

By examining the mechanism influencing fitness response of *C. parallelus* on food plant quality, diversity and food plant composition under laboratory and field conditions several studies, including the studies of this thesis encounter a variety of results. The questions of contradictory studies in this context easily arisen.

The influence of early feeding experiences has already been discussed as one mechanism deriving different results by studying grasshopper response of *C. parallelus* on plant species richness (see above the comparison between Chapter I and the study by Specht et al. 2008).

By disentangling questions concerning insect herbivore performance responses on food selection and diet both, laboratory experiments as well as field studies are important components of research. Whereas laboratory studies are conducted under an ideal but artificial environment which can standardize abiotic conditions (e.g. temperature and solar radiation) or exclude predation and competition, field studies assess the effect on the organisms in their natural environment making conclusions more relevant to natural conditions (Slansky & Rodrigues, 1987). In this relation, different fitness responses of *C. parallelus* populations due to varying plant species richness in the field (Chapter I) or plant species number offered in the diet (Chapter III) might be directly derived by differences of field and laboratory experimental designs. Although Chapter I showed a performance difference between grasshopper populations from different meadows in the study area due to plant species richness this was not verified by feeding *laboratory- reared* grasshoppers either on their *meadow diets* or even on a *standard diet* in the laboratory experiment (Chapter III). At least a pattern of the relationship discovered in Chapter I was shown in a correlation between grasshopper fecundity and plant species richness in *field-caught* grasshoppers fed on standard diet in the laboratory experiment (Chapter III) (published in Unsicker et al., 2010, using only grasshopper populations which had also been used in the correlative study in Chapter I). These differences between the studies in Chapter I and III might be due to the difference between field and laboratory studies. However, living and foraging in the field requires more than only nutrient balancing to reach an optimal diet as grasshoppers have to invest in anti-predation behavior, thermoregulation and much more. The constraints of living in the field influencing generalist insect herbivore performance, is at this point important to mention but was already discussed above. However, *laboratory-reared* grasshoppers living under optimal conditions in the lab have to work on maintaining performance even if this is not expressed in significant performance differences between the populations. Evidence for this statement is given in Franzke et al. (2010) in which the fitness response of one single *C. parallelus* population to different diets varying in plant species richness and composition was investigated. In this study the fitness response did not differ between grasshoppers fed with different diets but from data on food intake it was calculated that *C. parallelus* mixed food plants in different ways depending on the food plant identity and composition (Franzke et al., 2010). Gaining similar performance between *laboratory –reared* grasshoppers populations by feeding on different meadow diets (Chapter III) is assumed by active food plant selection, in this case this is only feasible under optimal conditions in the laboratory.

Anyway, the discovering of the impact of maternal effects in the field study where grasshopper populations have been relocated on different meadows (Chapter II) and the absence of these effects in the laboratory study by feeding nearly the same populations on *meadow* or *standard diet* (Chapter III) might also be caused by varying conditions in the lab versus the field, beside the point that the studies in Chapter II and III underlie really different experimental designs. The appearance of maternal effects described in the study of Chapter II could of course be aroused by relocating the grasshopper populations along a delta in certain *meadow attributes* (e.g. from parental meadows rich in plant diversity to *experimental meadows* low in plant diversity and vice versa).However, due to optimal living conditions in the laboratory it is assumed that also weak individuals match to reach a certain survival and fecundity whereas this would not happen in the field, even in cages were predation was excluded. These circumstances might also have accounted for masking maternal effects in the laboratory approach (Chapter III).

Interestingly, the book by Slansky & Rodriguez (1987) reviewed that: 'laboratory-reared insects are not necessarily of the same "quality" as field individuals (Chambers, 1977; King & Leppla, 1984)and thus it may not be appropriate to extrapolate the behavior of *laboratory-reared* individuals (as well as *field-caught* individuals studied in the laboratory) to field situations' (Slansky & Rodrigues, 1987). This statement must be kept in mind when discussing the chapters of the present thesis, as the results of the studies on *C. parallelus* do differ in the laboratory and in the field.

Coming back to the opening statement dealing with grasshopper performance responses to diet and the question if these studies are contradictory? After discussing the effect of early feeding experiences (see above) and differences in the study design in general as well as differences due to setting up laboratory and field studies in specific, I would clearly affirm that the results of the studies presented in this thesis are not contradictory because they have been derived under several different circumstances.

Perspective and Outlook - What do we know and what is still missing

The present thesis is underlining the importance of plant species richness, food plant quality and food plant composition on the performance of a generalist insect herbivore. At this point it is important to note that the findings concerning a generalist whereof not much studies and theories about the relationship of insect performance and plant diversity exists (Tscharntke et al., 2002, Franzke et al., 2010). The mechanism at work here was dietary mixing, which proposes a more complemented nutrient diet and /or a dilution of toxins by mixing differing food sources (Bernays et al., 1992;

Kaufmann, 1965; MacFarlane & Thorsteinson, 1980; Pulliam, 1975; Rapport, 1980). Nevertheless, by working on insect-plant relationships with real food plants under laboratory or field conditions it is important to keep in mind that even on short distances in the field striking differences in host preferences between insect populations exists (Schoonhoven et al., 1998)as well as large individual differences in host responses (Schoonhoven et al., 1998). This and the additional impact of 'other factors' like biotic and abiotic effects influencing herbivore performance in the field should be taken into account when valuing generalist insect herbivore responses on nutritional quality in the field. Furthermore this thesis is carving out the special position of food plant quality on generalist insect herbivore performance by stating the importance to combine food plant diversity, quality and composition with factors influencing insect herbivore performance indirectly due to changing food plant quality like abiotic factors.

The ability of mixing diets to maintain performance is also part of a whole research field in nutritional ecology working on macronutrients and artificial diets (Behmer, 2009; Bernays et al., 1992; Bernays et al., 1994; Chambers et al., 1995; Raubenheimer & Simpson, 2003; Waldbauer et al., 1984). Transferring the knowledge of nutritional ecology derived by discovering the self-regulatory abilities of insect herbivores into studies like presented in this thesis would help to gain further knowledge in both research areas, nutritional ecology as well as population ecology. This assumption, by the way, was just proposed by nutritional ecologists who suggested to design new synthesis by developing more comprehensive definitions of plant quality and how it varies with environmental conditions and furthermore develop collaborations across disciplines (e.g. physiological ecology, behavior and ecosystem sciences)(Behmer & Joern, 2012). Nevertheless, future studies in this context involving the study organism *C. parallelus* must take one step back, back into the laboratory. First of all it is of crucial importance to get to know the nutritional basics of this grasshopper species. Discovering the 'intake target' (the amount of macronutrients, mostly tested with carbohydrate and nitrogen, which has to be taken in to reach best performance) (Behmer, 2009; Raubenheimer & Simpson, 1993; Simpson & Raubenheimer, 1993) and learning more about the nutritional environments of the study area, where the different food plants along the gradient of plant species richness must be analyzed, is crucial for gaining new knowledge under field conditions. With this knowledge in the background it would be possible to open new doors regarding insect-plant relationships where for example not the relationship is of first interest rather the model system itself is used as basis to answer current ecological questions. To give but one recent example: The effect of changing climate could therefore be tested not only on a single plant- insect system but rather along a gradient of plant species diversity and composition on insect individuals or even on a community level, which would be a completely new approach.

Valuable new insights have been gained by finding maternal effects and early larval (feeding) experiences as well as the influence of biodiversity expressed through plant species richness on the performance of a generalist insect herbivore in field and laboratory conditions but it was furthermore accentuated that this picture could be shaped by including knowledge of nutritional ecology.

Summary

Satisfying their specific dietary needs, which are characterized by changing nutritional needs over a lifetime, is one of the biggest challenges for any animal. This includes insect herbivores that show a great variety in their responses to different aspects of food plant quality and therefore combine many interesting fields of ecological research, namely for example evolutionary, population, field and nutritional ecology. Generalist insect herbivores have a broad spectrum of potential food plants and are able to live in very different ecosystems including for example grasslands that largely differ in plant species richness and composition. Based on studies of dietary mixing it is assumed that generalist insect herbivores maintain or increase their performance by feeding on meadows with a high number of food plant species. Thus meadows with increasing plant species diversity and varying plant species composition promotes performance. The aim of my thesis was to disentangle the effect of plant species richness, composition and food plant quality on generalist insect herbivore performance in a combination of laboratory and field studies. Moreover, the effects of parental and early feeding experiences were analyzed. Finally, theoretical considerations about indirect effects of biotic and abiotic conditions on insect herbivore performance and the importance to combine knowledge of field and nutritional ecology integrate the current and other work into a review.

The meadow grasshopper *Chorthippus parallelus* served as study organism for the presented thesis because this species has a broad spectrum of food plants and its diet includes both, grasses and legumes.

Furthermore, it is one of the most abundant grasshopper species in diverse meadow habitats throughout Europe and plays as important herbivore a considerable role in grasslands. The studies have been conducted on or with food plants of meadows along a plant species diversity gradient within the biodiversity experiment BIOLOG (BIOLOgical diversity and Global change; funded by the BMBF 2000 -2010), located at the border between Thuringia and Bavaria in Central Germany.

Females of *C. parallelus* were collected in 15 meadows along a plant species richness gradient to analyze the oviposition rate and number of offspring. This observational study showed that grasshopper fecundity was positively affected by plant species richness. Moreover, the abundance of *C. parallelus* was positively correlated with plant species richness, plant community composition and solar radiation between different meadows in the study area. It is assumed that the positive relationship between performance and increased plant diversity is due to the possibility of dietary mixing, as with higher plant diversity *per se* also the number of possible food plants increases. Furthermore this study reveals the threat of biodiversity loss even on generalist insect herbivores.

A relocation experiment where grasshopper populations from different meadows were transferred and caged on meadows along a gradient of plant species richness and composition revealed a strong

influence of parental experiences on offspring performance, measured as survival and fecundity. The offspring of parents who lived on meadows high in plant species richness and low in biomass showed a better performance even if the relocated offspring has been reared in less favorable environments. Here again dietary mixing might serve as explanatory mechanism for the positive relationship between parental performance and higher plant species richness. However, this study gave a hint for strong maternal effects.

By feeding different grasshopper populations on a diverse *meadow diet* versus a two-species *standard diet*, strong effects of grasshopper origin reared either in the laboratory or caught in the 3rd to 4th instar in the field were revealed. *Laboratory-reared* grasshopper populations showed strong performance advantages when feeding on the diverse *meadow diet* compared to the *standard diet* and again dietary mixing theory might serve as explaining mechanism. Also the importance of early larval (feeding) experiences was discussed as determinant of diet effects on later grasshopper developmental stages.

Overall, the results of these studies underline the importance of plant species richness, plant composition and food plant quality on the performance of the generalist insect herbivore *C. parallelus* and support dietary mixing as a prominent possible mechanism to explain the experimental results. Nevertheless, these three factors alone were discussed to be no sufficient description of what influences generalist insect herbivore performance in the field. However, to reach a more mechanistic understanding of this plant- insect relationship or more specific the relation between insect performance and food plant quality the implication of 'Nutritional Ecology' would be extremely valuable. More specific information on the nutritional requirements of the herbivore (intake target point, amounts and proportion of macronutrients ingested) as well as information about the herbivores nutritional environment could help to disentangle the performance-relevant parameters for generalist insect herbivores foraging under field conditions. In a first step, it is necessary to gain information about the herbivores' nutritional requirements by feeding them on artificial diets and real food plants in the laboratory. In a second step, this information could be applied in the field where the nutritional value of the natural environment should also be studied. Finally, it is important to monitor additional factors like biotic (e.g. food plant chemical and biomechanical properties, predation) and abiotic (e.g. temperature, irradiation) conditions indirectly influencing the feeding behavior and insect performance by changing food plant quality. There is still much to discover about insect-plant relationships when the knowledge of field and nutritional ecology is combined in innovative studies using new technical approaches.

Zusammenfassung

Die Befriedigung der Nahrungsbedürfnisse, welche durch zeitlebens veränderliche Nährstoffbedürfnisse charakterisiert sind, stellt eine der alltäglichsten aber auch größten Herausforderungen für jedes Lebewesen dar. Dies betrifft auch herbivore Insekten, welche unterschiedlichste Reaktionen auf Futterpflanzenqualität zeigen und damit verschiedene, interessante Forschungsfelder der Ökologie miteinander kombinieren. Herbivoren Insekten, die an mehreren Arten einer oder mehrerer Pflanzenfamilien fressen(generalistische Lebensweise), ist es aufgrund des erweiterten Futterpflanzenspektrums möglich in verschiedenen Wiesenhabitaten mit sowohl unterschiedlichem Pflanzenartenreichtum, als auch unterschiedlicher Pflanzenartenkomposition zu existieren. Basierend auf Studien, die der „dietary mixing" Hypothese (beschreibt das Mischen von Futterpflanzen) unterliegen, ist anzunehmen, dass herbivore Insekten generalistischer Lebensweise ihre Fitness erhalten oder gar verbessern können, wenn sie auf Wiesen mit einer erhöhten Anzahl an Pflanzenartenfressen. Eine hohe Pflanzenartenzahl bzw. eine damit einhergehende veränderliche Pflanzenartenkomposition bedingt daher eine größere Anzahl von Futterpflanzenarten in einem Habitat.

Das Ziel der vorliegenden Arbeit war es, die Auswirkung von Pflanzenartenreichtum, Pflanzenartenkomposition, und Futterpflanzenqualität auf die Fitness von herbivoren Insekten mit generalistischer Lebensweise, sowohl in Labor, als auch in Feldstudien zu untersuchen.

Ein Hauptaugenmerk im experimentellen Teil der Arbeit lag dabei auf den Fraßerfahrungen früher Larvenstadien und den Erfahrungen der Elterngeneration, in Bezug auf Nahrung und Habitat, sowie deren Einflüsse auf die Fitness herbivorer Insekten. Ein Übersichtsartikel am Ende der vorliegenden Dissertation beleuchtet den durch veränderte Futterpflanzenqualität einhergehenden indirekten Einfluss von biotischen und abiotischen Faktoren auf die Fitness herbivorer Insekten. Darüber hinaus wird diskutiert, wie wichtig das Wissen von Nährstoff- und Feldökologie sowohl unter Einbezug der vorliegenden Arbeiten als auch der anderer Studien ist, und vor allem welche Bedeutung der Kombination beider Forschungsgebiete für zukünftige Studien hat. Als Studienobjekt diente die gemeine Feldheuschrecke *Chorthippus parallelus,* deren Speiseplan hauptsächlich Gräser aber auch Leguminosen beinhaltet. Zudem ist die Feldheuschrecke *C. parallelus* eine der abundantesten Arten in diversen Wiesenhabitaten Mitteleuropas, und darüber hinaus auch ein bedeutendes herbivores Insekt in Grünländern. Die experimentellen Studien wurden in Grasländern oder mit Pflanzen von Grasländern entlang eines pflanzlichen Diversitätsgradienten im Thüringer Schiefergebirge/Frankenwald im Rahmen des Biodiversitätsprojekts BIOLOG (BIOLOgical diversity and Global change, von 2000-2010 gefördert durch das Bundesministerium für Bildung und Forschung) durchgeführt.

Auf 15 Wiesen entlang eines Gradienten pflanzlichen Artenreichtums wurden Heuschreckenweibchen gefangen und im Labor zur Eiablage gebracht, wobei sowohl die Anzahl der Eipakete als auch die Anzahl der Nachkommen pro Weibchen als Fekunditätsmaße dienten. Es wurde gezeigt, dass Heuschreckenfitness aber auch die Abundanz von *C. parallelus* positiv mit einer erhöhten Pflanzenartenzahl auf den Heimatwiesen korreliert ist. Aber auch unterschiedliche Pflanzenartenkomposition und Sonneneinstrahlung zwischen den verschiedenen Wiesen beeinflussen die Heuschreckenabundanz. Eine Erklärung zwischen steigender Heuschreckenfitness und erhöhter Pflanzenartenzahl liegt der dietary mixing Hypothese zu Grunde, unter der Annahme, dass mit steigender Pflanzenartenzahl in einem Habitat auch die Zahl der potentiellen Futterpflanzenarten steigt. Des Weiteren offenbart die Studie eine Bedrohung durch Pflanzenartenverlust auch für generalistisch lebende Insektenherbivore.

Ein Umsiedlungssexperiment, bei dem verschiedene Heuschreckenpopulationen auf unterschiedlichen Wiesen entlang eines Gradienten pflanzlichen Artenreichtums mit variierender Pflanzenkomposition in Käfige versetzt wurden, zeigte starke elterliche Einflüsse auf die Fitness der Heuschreckenpopulationen. In diesem Zusammenhang konnte man beobachten, dass Eltern von besonders pflanzenartenreichen Wiesen bzw. Wiesen mit geringer Biomasse besonders fitte Nachkommen hervorbrachten, unabhängig davon, ob die Nachkommenschaft ebenso günstige Umweltbedingungen erfuhren, oder nicht . Auch in diesem Fall kann die dietary mixing Hypothese als erklärender Mechanismus für die positive Fitnessreaktion der Elterngeneration, und somit auch der nachkommenden Generation, aufgrund erhöhter Pflanzen- und Futterpflanzenartenzahl herangezogen werden. Die Studie zeigt, dass auch im Fraßverhalten sehr anpassungsfähige Herbivore wie *C. parallelus* stark durch maternale Effekte beeinflusst werden können.

In einem Laborexperiment mit verschiedenen Heuschreckenpopulationen, die entweder im Labor gezüchtet oder im dritten bis vierten Larvenstadium im Freiland gefangen wurden, traten starke Fitnessunterschiede als Reaktion auf unterschiedliche Diäten zwischen den verschiedenen Herkünften (Laborheuschrecken versus Freilandheuschrecken) auf. In diesem Zusammenhang zeigten Laborheuschrecken eine größere Fitness, wenn sie ein Leben lang auf einer artenreichen Wiesendiät, statt auf einer 2-Arten-Standarddiät fraßen, wobei hier die positive Fitnessreaktion erneut auf die Vorteile des Mischens von Futterpflanzen (dietary mixing) basiert. Die Auswirkungen der Fraßerfahrung früherer Larvenstadien auf die Fitness in der späteren Heuschreckenentwicklung wurden in den Ergebnissen der Studie besonders diskutiert.

Die experimentellen Studien der Arbeit betonen die Bedeutung von Pflanzenartenreichtum,– komposition, sowie von Futterpflanzenqualität für die Fitness des Insektenherbivoren *C. parallelus*, wobei der positiven Reaktion durch das Mischen von Futterpflanzen (dietary mixing) eine besondere Rolle zugeschrieben wird. Dennoch wurde dargelegt, dass diese drei Faktoren als alleinige Erklärung

für die Fitness generalistischer Insektenherbivore im Feld nicht ausreichend sind. Es wurde diskutiert, dass die Ergebnisse der Studien möglicherweise noch transparenter wären, wenn darüber hinaus spezifische Informationen bezüglich des Nährstoffhaushaltes bzw. der Nährstoffanforderungen des Studienobjekts (z.B. intake targe point, aufgenommene Mengen und Verhältnisse an Makronährstoffen) und der Nährstoffverfügbarkeit in seiner Umwelt vorlägen. Die Expertise des Forschungsfeldes der Nährstoffökologie könnte an diesem Punkt helfen weitere wichtige Informationen über die Fitness herbivorer Insekten unter Freilandbedingungen zu sammeln. Eine Voraussetzung dafür wären genaue Kenntnisse über die Nährstoffanforderungen des Herbivoren, welche man im Labor mit Hilfe von künstlichen Diäten aber auch beim Fressen an Futterpflanzen ermitteln kann. Die so gewonnen Informationen könnten später im Freiland angewandt werden. Natürlich nur unter der Voraussetzung, dass die Nährstoffverfügbarkeit der Umgebung des Herbivoren bekannt ist. In diesem Zusammenhang wurde die Bedeutung von biotischen (z.B. chemische und biomechanische Eigenschaften der Futterpflanzen, Prädation) und abiotischen (z.B. Temperatur, erhöhte CO_2-Werte) Faktoren auf Futterpflanzenqualität, und somit indirekt auch auf die Fitness von Insektenherbivoren im Detail erläutert und diskutiert.

Abschließend bleibt festzustellen, dass eine Kombination aus Nährstoff– und klassischer Feldökologie, unter Einbezug von innovativen Studien und neuen Technologien, ein Garant für neue zukünftige Entdeckungen in der Beziehung zwischen Pflanzen und Insekten ist.

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Curriculum Vitae

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10/2010 – 02/2011	**Freelancer for the Max-Planck-Institute of Biogeochemistry, Jena** Title: Conceptualisation, implementation and extension of a biodiversity ontology within the BExIS framework
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Education

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Juliane Heimann

Publications

Peer reviewed articles

Specht, J., Scherber, C., Unsicker, S. B., Köhler, G., Weisser, W. W. (2008). Diversity and beyond: plant functional identity determines herbivore performance. Journal of Animal Ecology, 77(5), 1047-1055.

Franzke, A., Unsicker, S. B., **Specht, J**., Köhler, G., Weisser, W. W. (2010). Being a generalist herbivore in a diverse world: how do diets from different grasslands influence food plant selection and fitness of the grasshopper *Chorthippus parallelus*? Ecological Entomology, 35, 126-138.

Scherber, C., **Heimann J.**, Köhler G., Mischunas N., Weisser W. W. (2010). Functional identity versus species richness: herbivory resistance in plant communities. Oecologia 163, 707-717

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Other articles

Köhler, G., Pfeiffer, S., **Specht, J**., Wagner, M. (2005) Neu entdeckte Populationen von Ödlandschrecken (Caelifera: Acrididae, Oedipodinae) im Muschelkalkgebiet um Jena / Thüringen. Thüringer Faunistische Abhandlungen, 137-147.

Heimann, J., Wiehle, C., Weisser, W. (2010). Die Wiese – Artenvielfalt vor der Haustür erleben. Beitrag zur BIOLOG Abschlussveranstaltung ‚Biodiversitätsforschung – Meilensteine zur Nachhaltigkeit' . Berlin März 2010

http://www.biolog-veranstaltung.de/pdf/heimann_ua_schulprojekt_biolog.pdf

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Congress contributions

Talk presentation

Specht, J. , Unsicker S.B. , Weisser W.W. (2007) A test for local adaptation in the generalist grasshopper *Chorthippus parallelus* (Acrididae). Tagung der Deutschen Gesellschaft für allgemeine und angewandte Entomologie e.V. (DGaaE) 26.02 – 01.03.2007, Innsbruck

Specht, J. , Unsicker S.B., Weisser W.W. (2007) Origin and diet affect the performance of local population of the grasshopper *Chorthippus parallelus* – a transplantation experiment. Jahrestagung der Gesellschaft für Ökologie (GFÖ) 10.09 – 14.09.2007, Marburg

Heimann, J., Unsicker S.B., Weisser W.W. (2010) The performance of a generalist herbivore in a diverse world – Plant species richness, composition and quality matters. Jahrestagung der Gesellschaft für Ökologie (GFÖ) 30.08 – 03.09.2010, Gießen

Poster presentation

Heimann, J., Unsicker S.B., Weisser W.W. (2009) The effect of food composition on the fitness of grasshopper populations. Tagung der Deutschen Gesellschaft für allgemeine und angewandte Entomologie e.V. (DGaaE) 16.03. – 19.03.2009, Göttingen

Selbstständigkeitserklärung

Hiermit erkläre ich, Juliane Heimann, dass ich die vorliegende Dissertation mit dem Titel **" Generalist insect herbivore performance in dependence of plant species richness, composition and quality"** selbstständig und nur unter Verwendung der angegebenen Hilfsmittel und Literatur angefertigt habe.

Jena, April 2012   …………………………………….

                                                              Juliane Heimann