

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*?

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Abstract. 1. Generalist insect herbivores occupy a variety of habitats that differ in food plant composition. Dietary mixing has been proposed as a possibility for generalists to overcome nutritional deficiencies of single plant species, but only a few studies have investigated herbivore feeding and fitness for diets that resemble natural scenarios. We studied feeding behaviour, survival, and reproduction of the generalist grasshopper *Chorthippus parallelus* raised on food plants of four typical habitats.

2. Grasshopper diet consisted of grasses (92.5%), legumes (6.7%) and, in small quantities, other forbs (0.8%). Diet selection differed between the four food plant mixtures, and depended on grasshopper sex and developmental stage. There was no correlation between the relative abundance of plant species in the field and the fraction of these species in the grasshopper diet.

3. Grasshoppers survived on average for 40.4 ± 1.0 days before maturity, grew 106.8 mg until maturity moult, and females laid 4.1 ± 0.4 egg pods, each of which contained 8.5 ± 0.4 eggs. However, despite the differences in feeding behaviour, grasshopper fitness was the same in all of the four food plant mixtures. While the digestibility of ingested food was similar in the four different treatments, indices indicated differences in the conversion efficiency to body mass.

4. Our results show that *C. parallelus* is a plastic feeder with no fixed preferences in diet composition. The results emphasise that generalist herbivores can counteract putative quality deficiencies of single food plants by selective dietary mixing.

Key words. Dietary mixing, feeding plasticity, fitness, habitat quality, insect ecology.

Introduction

Habitats of herbivorous insects often differ in quality, such that survival and reproduction of individuals vary among the habitats. One important component of habitat quality for herbivores is the quality and quantity of food plants available. While a large number of studies have shown that differences in food plant availability among habitats affect herbivore performance,

most of these studies have been conducted with specialist herbivores where low food plant quality or the lack of a particular plant species often results in a severe decrease in herbivore fitness (e.g. Singer & Thomas, 1996; Hanski & Singer, 2001). In contrast, generalist herbivores that feed on a wide range of plant species may suffer less from changes in food plant composition, but studies on the performance of generalist herbivores in different habitats are rare (Tscharrantke *et al.*, 2002).

One mechanism that has been put forward for generalist herbivores to cope with food plant species of low quality is dietary mixing (Bernays & Bright, 1993; Haegele & Rowell-Rahier, 1999; Miura & Ohsaki, 2004a; Berner *et al.*, 2005; Mody *et al.*,

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2007; Unsicker *et al.*, 2008). Dietary mixing is thought to benefit herbivores by allowing either a more balanced intake of nutrients ('nutrient complementation hypothesis', (Pulliam, 1975; Rapport, 1980; Lee *et al.*, 2002; Berner *et al.*, 2005; Takeuchi *et al.*, 2005) or by 'toxin dilution', whereby individual toxins produced by particular plant species are consumed in lower dosage in broader diets which lessens their effect (Free-land & Janzen, 1974; Behmer *et al.*, 2002; see review by Singer *et al.*, 2002; Marsh *et al.*, 2006). More recently, Raubenheimer and Simpson have developed a 'geometric model' of feeding for generalist insects that allows an exploration of how the intake of dietary protein, carbohydrate and salts affects herbivore development (see review by Raubenheimer & Simpson, 1999), and based on this approach, a number of studies have shown that herbivores generally have to balance the intake of different nutrients for successful development (Behmer & Joern, 1993; Lee *et al.*, 2002; Berner *et al.*, 2005). A corollary of dietary mixing theory is that it allows generalists to reach similar fitness in habitats with different food plant compositions. Most studies testing the theory have been conducted under laboratory conditions. Even there, however, dietary mixing may not always compensate for any shortage in the quality of individual plants. For example, Berner *et al.* (2005) showed for the grasshopper *Omocestus viridulus* that individuals can counterbalance N-shortage by compensatory feeding, and are capable of selectively feeding among grasses of contrasting nutritional quality when given a choice, yet grasshopper adult weight decreased when plant nitrogen levels were low despite compensatory feeding. In general, little is known about the ability of generalist insect herbivores to compensate for large differences in host plant composition and quality.

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). Among these acridids, the meadow grasshopper *Chorthippus parallelus* (Zetterstedt, 1821) is one of the most common species throughout Central Europe (Köhler & Schaller, 1981; Ingrisch & Köhler, 1998; Maas *et al.*, 2002). In Central Germany, the species occurs in a variety of grassland types differing greatly in plant species composition (Ingrisch & Köhler, 1998). Earlier studies on feeding preferences of *C. parallelus* have shown that while its diet mainly consists of grasses (Poaceae), legumes and other forbs are also consumed in variable proportions (Kaufmann, 1965; Bernays & Chapman, 1970a, 1970b; Köhler & Schaller, 1981; Gardiner & Hill, 2004; Unsicker *et al.*, 2008; Unsicker *et al.*, 2009). For a number of acridid grasshoppers including *C. parallelus*, positive effects of diet mixing on fitness parameters have been demonstrated (e.g. Uvarov, 1977; Bernays & Bright, 1993; Bernays & Minkenberg, 1997; Miura & Ohsaki, 2004a; Unsicker *et al.*, 2008). Unsicker *et al.* (2009) found in a correlational study that differences in grasshopper abundances and fitness between different study sites were positively correlated to plant species richness in the sites (Unsicker *et al.*, 2009). The available studies show that diet mixing principally allows acridid grasshoppers to compensate nutritional deficits or toxicity in any one plant species, for example, but they do not answer the question of whether

dietary mixing allows grasshoppers to reach similar fitness in habitats differing in plant species composition.

In this study we tested the performance of *C. parallelus* when offered host plants from four different grasslands with very different plant species composition. Grasshoppers were kept individually and their feeding behaviour and performance were followed from birth to death. We asked three questions. (i) Are there differences in diet selection when different plant species combinations are offered? (ii) Do host plant preferences depend on grasshopper developmental stage and sex? (iii) How do different food supplies from different meadows affect the survival and reproduction of *C. parallelus*?

Materials and methods

Study organism

Chorthippus parallelus is the most abundant grasshopper species in our study area in Central Germany (Köhler, 2001; Köhler & Renker, 2004; Pratsch, 2004). In August 2004 adult grasshoppers of *C. parallelus* were caught by hand or sweep-net in an extensively managed meadow ('Jena') close to the city of Jena, Germany (50°56'N, 11°37'E). The grasshoppers were kept in groups of about 25 individuals in plastic terraria (46 × 19 × 34 cm) in the laboratory for 14 days, not separated by sex. Each terrarium contained two plastic cups with a 10 cm diameter, filled with a moist mixture of sand and garden soil (1:1) for oviposition. In October the plastic cups that contained egg pods were transferred to the refrigerator where they were stored at 5 °C until the start of the experiment in July 2005.

Grasshopper diets

Four different meadows were chosen to test the effects of different plant species combinations on the fitness of *C. parallelus*. In the remainder of this paper, these four meadows and the food plant mixtures (= treatments) originating from them will be named according to the village/town that is closest to them (Table 1). Plant communities in the selected sites ('Jena', 'Schlegel', 'Tschirn', 'Steinbach a. W.') differed in plant species richness and plant community composition (Appendix 1). The meadows (except 'Jena') were situated in the Thüringer Schiefergebirge and Frankenwald, a low mountain range at the Bavarian/Thuringian border in Central Germany. All meadows in the Schiefergebirge are part of a biodiversity research project where meadows were selected along a gradient in plant species richness (Kahmen *et al.*, 2005; Unsicker *et al.*, 2006). 'Tschirn', and 'Steinbach a. W.' are species-rich mountain meadows in the Frankenwald, and the 'Schlegel' site is a species poorer fodder meadow in the Thüringer Schiefergebirge (Table 1). All meadows are extensively managed, i.e. they are mown twice a year and generally not fertilised. The 'Jena' site was similar to the two species-rich sites in terms of richness. In the framework of the DIVA-Jena research project, vegetation relevés were taken every year and showed that year-to-year variation in plant species composition is very small, such that our selection of plant species can be

Table 1. Description of study sites and food plant mixtures used in the experiment.

	'Jena'	'Schlegel'	'Tschirn'	'Steinbach a. W.'
Position	50°56'N, 11°37'E	50°24'N, 11°37'E	50°23'N, 11°26'E	50°26'N, 11°24'E
Altitude (m NN)	140	641	606	678
Species density (plants m ⁻²)	26	13	24	29
Grasses	<i>Arrhenatherum elatius</i> 13% <i>Agropyron repens</i> 8% <i>Agrostis canina</i> 2% <i>Bromus erectus</i> 8% <i>Dactylis glomerata</i> 10% <i>Deschampsia cespitosa</i> 2% <i>Festuca rubra</i> 3% <i>Phleum pratense</i> 1%	<i>Dactylis glomerata</i> 13% <i>Lolium multiflorum</i> 8% <i>Lolium perenne</i> 2% <i>Phleum pratense</i> 8%	48% <i>Agrostis tenuis</i> 2% <i>Anthoxanthum odoratum</i> rare <i>Dactylis glomerata</i> rare <i>Festuca rubra</i> <i>Holcus lanatus</i> <i>Phleum pratense</i> <i>Trisetum flavescens</i>	1% <i>Agrostis tenuis</i> 11% 4% <i>Anthoxanthum odoratum</i> 2% 1% <i>Dactylis glomerata</i> 2% 32% <i>Festuca rubra</i> 30% rare <i>Holcus mollis</i> rare rare <i>Phleum pratense</i> rare 4% <i>Trisetum flavescens</i> 5%
Number of grasses	8	4	7	7
Legumes	<i>Trifolium pratense</i>	<i>Trifolium pratense</i> 2% <i>Trifolium repens</i>	2% <i>Trifolium pratense</i> 1% <i>Trifolium repens</i>	21% <i>Trifolium pratense</i> 2% 4%
Number of legumes	1	2	2	1
Forbs	<i>Achillea millefolium</i> 3% <i>Galium mollugo</i> 5% <i>Geranium pratense</i> 20% <i>Pimpinella major</i> 2% <i>Potentilla reptans</i> 10% <i>Veronica chamaedrys</i> 2%	<i>Achillea millefolium</i> 3% <i>Chaerophyllum aureum</i> rare <i>Hypericum maculatum</i> rare <i>Taraxacum officinale</i> 7% <i>Veronica chamaedrys</i> 3%	1% <i>Alchemilla vulgaris</i> rare <i>Hypericum maculatum</i> rare <i>Plantago lanceolata</i> 7% <i>Veronica chamaedrys</i>	4% <i>Achillea millefolium</i> 3% 4% <i>Alchemilla vulgaris</i> 3% 7% <i>Centaurea pseudophrygia</i> 10% 4% <i>Geranium sylvaticum</i> 19% rare <i>Hypericum maculatum</i> rare <i>Plantago lanceolata</i> 7% <i>Taraxacum officinale</i> 1%
Number of forbs	6	5	4	7
Total number of plant species	15	11	13	15

Percentages are mean cover percentages based on relevés of 1 m² (11 for 'Schlegel', 'Tschirn', and 'Steinbach a. W.' and six for 'Jena'). 'Rare' denotes plant species that had <1% cover in relevés but occurred at higher percentages when the entire meadow was searched for potential food plant species. Bold parts of plant names indicate acronyms of plant names. NN is altitude m.a.s.l.

considered a representative for the different meadows (Stein et al., 2009).

For each selected meadow, we proceeded as follows to select plant species for the food plant mixture: (i) all grass species were included in the food plant mixture independent of their relative abundance, (ii) forbs were selected if their mean cover in the grassland exceeded 1% based on plant cover estimates from 11 × 1 m²—relevés obtained in 2005, (iii) forbs with cover percentage of <1% in 2005 were also selected when a survey at the end of June 2005 over the entire meadow indicated that their cover on the field scale was above 1%, (iv) *Meum athamanticum*, *Phyteuma spicatum*, *Polygonum bistorta*, and *Rhinanthus minor* satisfied the second criterion, but were not included in the experiment as they are generally avoided by *C. parallelus* based on preliminary experiments (S. B. Unsicker, pers. obs.). Table 1 lists the plant species selected for each treatment.

Experimental set-up

In mid-July 2005, egg pods were removed from the refrigerator and put in groups of two into plastic cups filled with a mixture of moist sand and soil (1:1). The cups were covered with mesh (width 1 mm) to prevent hatching grasshopper nymphs from escaping, and placed in the laboratory at ambient temperature (around 25 °C). Hatching

started on 29 July 2005 and most of the grasshoppers hatched within 2 weeks. Freshly hatched grasshoppers were separated in groups of 60 individuals into 6-litre plastic containers (fauna box: 155 × 155 × 250 mm, Savic, Heule, Belgium) where they were fed with freshly cut wheat leaves [*Triticum aestivum* (L.)] until the beginning of the experiment for a maximum of 4 days after hatching.

One hundred and twenty grasshopper nymphs were randomly assigned to the four feeding treatments with 30 replicates per treatment. All grasshoppers were reared individually in 6-litre plastic containers (fauna box, Savic) from the first instar to death, and cages were inspected every 4 days (see below). The containers were placed outdoors at the Remderoda field station of the Institute of Ecology in Jena on a wooden shelf-unit (6.20 × 2.30 × 2.00 m). In order to account for microclimatic variation, containers were placed onto the shelves in 30 groups (blocks), each of which consisted of one container of each of the four different feeding treatments. The position of the containers within a block was rotated every fourth day when cages were inspected. On sunny days, white cotton sheets were fixed on the sunny side of the shelf unit to shade containers and to avoid excessive heat. The first grasshoppers were placed into containers blockwise starting on 29 July 2005 until 1 August 2005, and the experiment lasted three months until 6 November 2005. Grasshoppers that

died during the first 4 days of the experiment were replaced but are not included in the present analyses.

The food plants for the feeding treatments were harvested weekly in each of the four meadows. Plants were cut 1 cm aboveground and stored in zip-lock bags until use in the fridge. To offer the plants to the grasshoppers, a plastic box (115 × 115 × 60 mm) filled with water and covered with aluminium mesh and cling film was placed into each container. Cling film above the aluminium mesh was used to prevent the grasshopper from drowning and to prevent the loss of grasshopper faeces. Food plants were offered *ad libitum* as leaves, and placed with the petiole through the cling film and mesh into the plastic box. Every fourth day the amount of plant material offered to the grasshoppers was quantified and templates were drawn on paper for each individual species and for various leaf sizes. We exchanged old leaves of the particular food plants by new leaves every 4 days. The number of leaves of each size class introduced into a container was noted for each plant species at each inspection day.

Estimation of food consumption

Every fourth day when food plants were exchanged, food consumption over the last 4 days was quantified. For completely consumed plants the templates were used to estimate the leaf area eaten by the grasshopper. For partly eaten leaves, the leaf area removed was quantified (in mm²) using graph paper, whereby the paper templates served to reconstruct original leaf size if necessary. At the end of the experiment, leaf area eaten was converted into biomass consumption. This was done by determining the dry weight per square centimetre leaf area for all food plants and meadows. Discs (area 1 cm²) were cut from 10 individuals of each species of each meadow, dried for 24 h at 70 °C to obtain constant dry weights of the plant tissue (Köhler *et al.*, 1987) and weighed. The conversion factor for each plant species for each food plant mixture was the average of the 10 measurements.

Faeces were collected after each moulting and at the end of the experiment. Faeces were dried for 24 h at 70 °C and weighed.

Grasshopper fitness and development

At each inspection of the grasshopper containers (every 4 days), we noted if the individual was still alive and whether it had moulted into the next developmental stage N2–N4 (second to fourth instar). For males, the experiment ended after maturity moult when their development time was noted and they were used to mate with females. To include males into our survival analysis, we calculated survival until maturity as the number of days until individuals died or reached maturity, whatever came first. Single males were placed into a female container of the same food plant treatment after female maturation. Males were kept together with females for 8 days. During this time, food consumption data could not be collected, as consumption rates could not be assigned to individuals. Females continued to be fed and observed until the end of their life or until day 100

of the experiment, whatever came first. The experiment was terminated at day 100 after the start when there were still 23 females alive.

Fresh weight of all 120 grasshoppers was measured in the first instar, just before the experiment started, after moulting into the fourth nymphal instar and after maturity moult. At the end of the experiment, surviving grasshoppers were stored in 70% ethanol and later dried at 70 °C for 24 h to constant weight. Individuals that died before the end of the experiment were also placed in ethanol and weighed.

For oviposition, each female grasshopper was provided with a small plastic tub filled with a moistened sand/soil mixture (1:1). After the termination of the experiment the egg pods laid by each female were counted, weighed and buried in the soil again. All egg pods were overwintered in the refrigerator at 8 °C. To determine the hatching rate, egg pods were incubated for 4 weeks at ambient room temperature (26.6 °C on average) starting 9 May 2006. All hatchlings were counted.

Food utilisation efficiency

Utilisation indices and growth rate were calculated for each individual grasshopper according to Waldbauer (1968).

Digestibility. The *approximate digestibility* (AD) was calculated at 4 days after maturity moult as:

$$AD = [(dry\ weight\ of\ ingested\ food - dry\ weight\ of\ faeces) / dry\ weight\ of\ ingested\ food] \times 100.$$

Conversion of ingested food. The *efficiency of conversion of ingested food to body substance* (ECI) was calculated at day 4 after maturity moult as:

$$ECI = (grasshopper\ dry\ weight\ at\ death / dry\ weight\ of\ ingested\ food) \times 100.$$

Conversion of digested food. The *efficiency with which digested food is converted to body substance* (ECD) at day 4 after maturity moult was calculated as:

$$ECD = [grasshopper\ dry\ weight\ at\ death / (dry\ weight\ of\ ingested\ food - dry\ weight\ of\ faeces)] \times 100.$$

ECI and ECD at maturity were calculated using the dry weight of individuals after death, because we did not sacrifice individuals during the experiment. For females this was the dry weight at the end of the experiment and for males the dry weight after they were removed from the female cages (see above), about 8–12 days after maturity moult.

Growth rate. The *relative growth rate* (GR) was calculated as $GR = G/T$ where G is the fresh weight gain of animals from the start of the experiment to fourth instar or to maturity, and T is the length of the feeding period in days.

Statistical analyses

To examine the effects of the food plant mixture on fitness measures and food utilisation indices, general linear models (Type III) were performed with SPSS 13.0. Repeated measures ANOVAs (rMANOVA) were used when the different nymphal instars of the grasshoppers were included in the same analysis. All statistical assumptions, such as normal distribution and homoscedasticity were checked and data were transformed if necessary. In cases where transformation did not restore normality and equal variances, nonparametric tests were used.

Results

Grasshopper survival

Of the 120 grasshoppers in the experiment, 87 individuals reached maturity. There was no difference among food plant mixtures in survival until maturity moult (χ^2 -test, $\chi^2 = 4.138$, d.f. = 3, $P = 0.247$).

Average survival until maturity moult of *C. parallelus* grasshoppers was 40.4 ± 0.98 days (range 18–72 days) and did not differ between food plant mixtures (ANOVA: $F_{3,102} = 1.35$, $P = 0.264$; males: $F_{3,61} = 1.64$, $P = 0.190$, females: $F_{3,37} = 0.221$, $P = 0.881$; population means 'Jena': average 42.6 ± 2.1 days, males 44.2 ± 2.1 , females 38.6 ± 5.2 ; 'Schlegel': 37.1 ± 2.2 , 35.8 ± 2.8 , 38.1 ± 3.3 ; 'Tschirn': 40.6 ± 1.7 , 40.7 ± 2.1 , 40.4 ± 3.0 ; 'Steinbach a. W.': 41 ± 1.9 , 40.8 ± 2.8 , 41.3 ± 1.3).

Food consumption

Grasshoppers fed on all plant species that were offered in the different food plant mixtures (Appendix 1). Averaged over the life of all grasshoppers, grasses comprised 92.5%, legumes 6.7% and the remaining herb species 0.8% of the diet in terms of biomass (dry weight) consumed (Table 2). There were great differences among the different grass species in the amounts in which they were consumed. Some grass species such as *Anthoxanthum odoratum* and *Holcus mollis*

were consumed less than the legumes *Trifolium repens*, and in particular *Trifolium pratense* (Fig. A1).

The proportion consumed of legumes and other forbs differed among the treatments (rMANOVAs, proportions arcsin-transformed, legumes $F_{3,65} = 6.24$, $P = 0.001$; forbs $F_{3,65} = 4.06$, $P = 0.010$, Fig. 1, Table 2), but not in the proportion of grasses ($F_{3,65} = 2.22$, $P = 0.094$, Fig. 1, Table 2). While in the food plant mixture from 'Schlegel', legumes made up more than 10% of the diet throughout all nymphal stages, this proportion was less than 10% for the other three mixtures (except for nymphal stage 4 in the 'Tschirn' mixture, Fig. 1). There were differences between males and females in the proportion of legumes in the diet (rMANOVA, proportions arcsin-transformed, $F_{1,65} = 4.61$, $P = 0.036$, Fig. 2, Table 2) but for grasses and forbs there were no differences (grasses $F_{1,65} = 0.05$, $P = 0.825$; forbs $F_{1,65} = 0.45$, $P = 0.505$, Fig. 2, Table 2).

The preferred grass species differed among the treatments (Fig. A1). *Dactylis glomerata*, *Phleum pratense*, and *Trifolium pratense* were part of all four food plant mixtures and the proportion of these species in the diet differed among mixtures and between nymphal instars (*D. glomerata*: rMANOVA, treatment $F_{3,65} = 17.15$, $P < 0.0001$, sex $F_{1,65} = 0.33$, $P = 0.567$; *P. pratense*: rMANOVA, treatment $F_{3,65} = 5.74$, $P = 0.001$, sex $F_{1,65} = 0.07$, $P = 0.798$; *T. pratense*: rMANOVA, proportions arcsin-transformed, treatment $F_{3,65} = 12.81$, $P < 0.0001$, sex $F_{1,65} = 2.91$, $P = 0.093$, Fig. 3). For example, *D. glomerata* was preferred in the third nymphal instar and its overall consumption was highest in the 'Steinbach a. W.' treatment and least in the 'Jena' treatment (Fig. 3a,b). Feeding on *P. pratense* was highest in the 'Steinbach a. W.' treatment, but only until the third nymphal stage (Fig. 3c,d) while *T. pratense* was consumed most in the 'Schlegel' treatment throughout all developmental stages (Fig. 3e,f).

To test if grasshopper preferences mirror plant abundances in their meadow of origin, 'Jena', we correlated the rank order of species in the 'Jena' meadow (based on cover abundances) with the rank order of species in the experiment (based on consumption). The correlation was not significant ($P > 0.05$). Similar correlations between plant cover abundances and plant consumption rates for the other three food plant mixtures were also not significant.

Table 2. Total food consumption until maturity stage.

	Grasses (mg dw)		Legumes (mg dw)		Forbs (mg dw)		Total consumption (mg dw)	
	Males	Females	Males	Females	Males	Females	Males	Females
'Jena' m ($n = 13$), f ($n = 2$)	158.7 \pm 4.93	231.5 \pm 7.99	2.7 \pm 0.72	3.1 \pm 2.77	3.2 \pm 0.71	4.4 \pm 3.20	162.0 \pm 4.84	234.9 \pm 11.19
'Schlegel' m ($n = 5$), f ($n = 8$)	114.2 \pm 8.60	154.8 \pm 13.52	18.4 \pm 3.56	35.8 \pm 4.24	18.8 \pm 3.33	36.6 \pm 4.09	133.0 \pm 5.97	191.4 \pm 14.64
'Tschirn' m ($n = 15$), f ($n = 6$)	150.3 \pm 5.26	188.8 \pm 14.35	7.0 \pm 1.25	26.5 \pm 2.90	7.3 \pm 1.24	26.7 \pm 2.82	157.6 \pm 4.92	215.6 \pm 15.68
'Steinbach a. W.' m ($n = 12$), f ($n = 9$)	124.8 \pm 7.19	179.4 \pm 9.45	7.9 \pm 2.11	12.4 \pm 2.48	9.7 \pm 2.30	13.9 \pm 2.42	134.5 \pm 8.02	193.3 \pm 10.30

n is the number of individuals that reached maturity in each treatment.

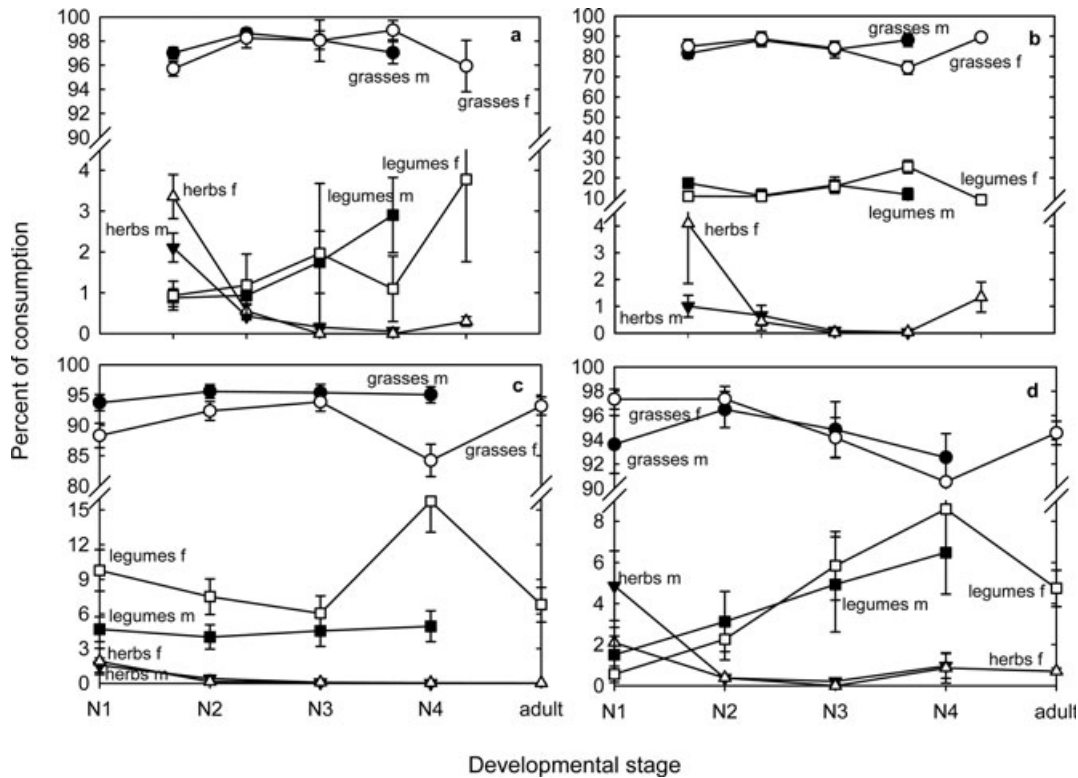


Fig. 1. The proportion of grasses, legumes, and other forbs in the diet of *Chorthippus parallelus* in each developmental stage for four plant species mixtures. Note scale differences on y-axes. m, male grasshoppers; f, female grasshoppers; N1–N4, nymphal stage of grasshopper. Bars represent means \pm SE.

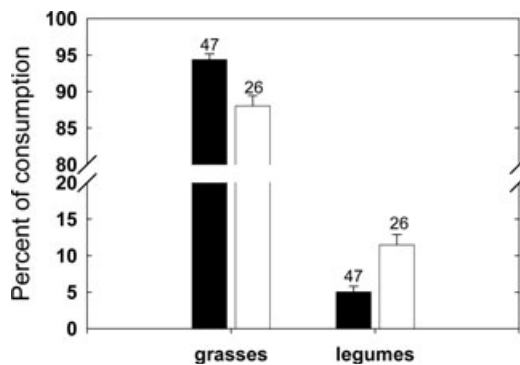


Fig. 2. The biomass proportion of grasses and legumes in the diet of male (full bars) and female (open bars) *Chorthippus parallelus* until maturity moult. Bars represent means \pm SE and numbers above bars give number of replicates.

Food utilisation efficiency

The digestibility of ingested food (AD) was calculated at the time of maturity moult and did not differ between the four food plant mixtures ($F_{3,66} = 1.28$, $P = 0.289$) or between sexes ($F_{1,66} = 3.41$, $P = 0.070$), and the interaction between food plant mixture and sex was also not significant ($F_{3,66} = 1.77$, $P = 0.163$, Fig. 4a). In contrast to AD, both the efficiency of conversion of ingested food to body substance

(ECI) and the efficiency with which digested food is converted to body substance (ECD), differed among food plant mixtures (ECI: food plant mixture $F_{3,66} = 5.79$, $P = 0.001$, sex $F_{1,66} = 186.11$, $P < 0.001$; ECD: food plant mixture $F_{3,66} = 3.25$, $P = 0.028$, sex $F_{1,66} = 92.14$, $P < 0.001$; Fig. 4b,c). The interaction between food plant mixture and sex was not significant for both ECI ($F_{3,66} = 1.35$, $P = 0.265$) and ECD ($F_{3,66} = 1.83$, $P = 0.151$). In general, ECI and ECD were highest in the 'Steinbach a. W.' treatment and lowest in the 'Jena' mixture (Fig. 4b,c).

Grasshopper growth

After hatching, grasshoppers weighed on average 6.9 ± 0.14 mg (males 6.9 ± 0.2 mg, females 7.0 ± 0.2 mg). At the beginning of the fourth nymphal instar, fresh weight had increased to 81.1 ± 2.4 mg (males 69.6 ± 1.5 mg, females 101.7 ± 3.7 mg) across the food plant mixtures, an average increase of 74.1 mg (males 62.7 mg, females 94.7 mg, Fig. 5a). Fresh weight in the fourth instar was significantly affected by the interaction treatment \times sex ($F_{3,66} = 4.32$, $P = 0.008$). In females, fresh weight was highest in the 'Steinbach a. W.' mixture and lowest in the 'Tschirn' plant mixture while the opposite was true for males (Fig. 5a).

Adult fresh weight after maturity moult was on average 113.8 ± 5.1 mg (males 85.0 ± 1.6 mg, females 167.9 ± 5.0 mg), which was an increase of 106.8 mg (males

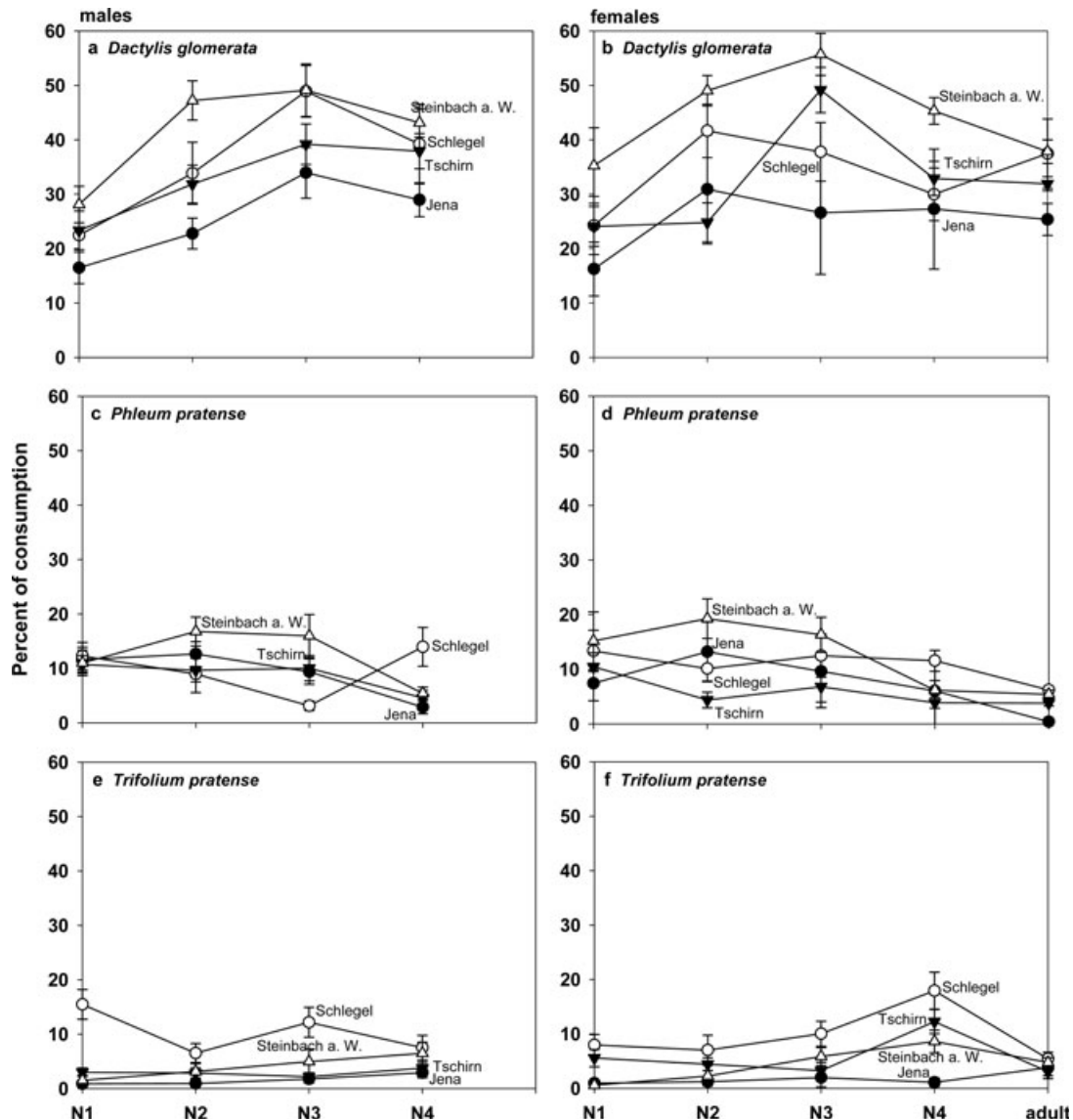


Fig. 3. Consumption of the grasses *Dactylis glomerata* and *Phleum pratense* and the legume *Trifolium pratense* by *Chorthippus parallelus* individuals in the experiment as a function of grasshopper sex, instar, and food plant mixture. N1–N4, nymphal stage of grasshopper. Bars represent means \pm SE.

78.1 mg, females 161.0 mg) since birth (Fig. 5b,c). Adult fresh weight was significantly higher in females ($F_{1,66} = 274.48$, $P < 0.001$), but there was no difference in adult fresh weight between the different food plant mixtures ($F_{3,66} = 1.22$, $P = 0.310$). The interaction between food plant mixture and sex was not significant ($F_{3,66} = 1.65$, $P = 0.186$, Fig. 5b). Growth rate was on average 2.4 ± 0.12 mg per day (males 1.8 ± 0.05 mg, females 3.7 ± 0.14 mg) from the introduction into the cage to maturity moult, and did not differ between food plant mixtures ($F_{3,66} = 2.11$, $P = 0.108$ Fig. 5c). Growth rate in females was significantly higher than in males ($F_{1,66} = 157.19$, $P > 0.001$, Fig. 5c). The interaction between food plant mixture and sex was marginally non-significant ($F_{3,66} = 2.57$, $P = 0.062$, Fig. 5c).

Grasshopper reproduction

Female grasshoppers laid on average 4.1 ± 0.4 egg pods during 46.8 ± 1.6 ($n = 28$) days of adulthood ($n = 28$ females). The number of egg pods laid per female did not differ between food plant mixtures ($F_{3,24} = 0.78$, $P = 0.515$, Fig. 6a). Each egg pod contained on average 8.5 ± 0.4 eggs and the number of eggs per pod was also independent of food plant mixture ($F_{3,24} = 0.78$, $P = 0.517$, Fig. 6c), despite significant differences in the fresh weight of egg pods ($F_{3,24} = 5.46$, $P = 0.005$, Fig. 6b).

From each egg pod on average 1.1 ± 0.28 grasshoppers hatched, an average hatching success of 13.2%. Dissection of the egg pods showed that most of the eggs were attacked by

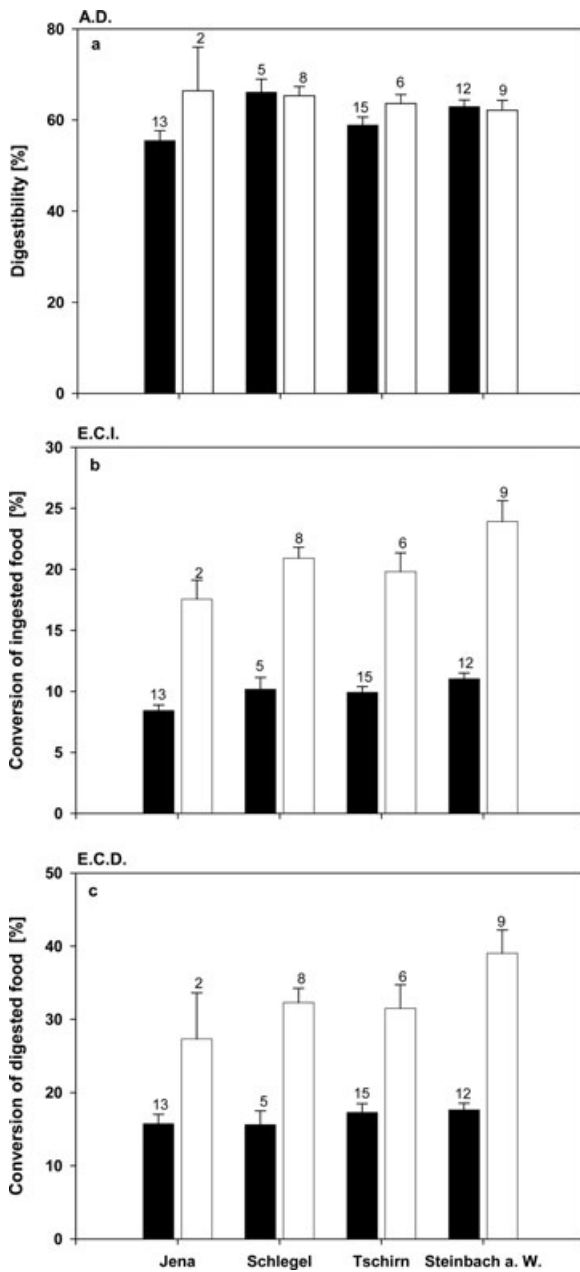


Fig. 4. Food utilisation efficiencies of male (full bars) and female (open bars) *Chorthippus parallelus* grasshoppers. (a) digestibility (AD), (b) conversion of ingested food (ECI), (c) conversion of digested food (ECD). Bars represent means \pm SE and numbers above bars give number of replicates. See text for explanation of indices.

fungus, were unfertilised or did not completely develop (782 eggs from a total of 963 eggs). Due to the low hatching success, data were not subjected to further analysis.

Discussion

The main result of our study is that individuals of *C. parallelus* had the same survival and fecundity when feeding

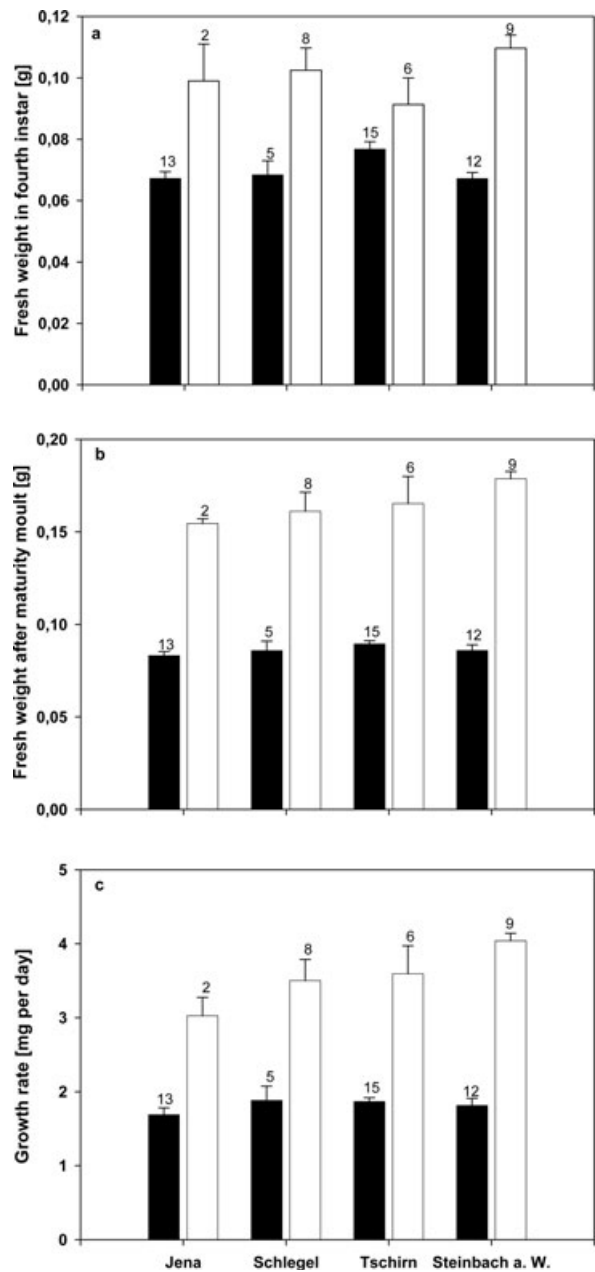


Fig. 5. Effects of food plant mixtures on (a) grasshopper weight after moulting into the fourth instar, (b) grasshopper weight after maturity moulting, and (c) growth rate from birth until maturity moulting, for male (full bars) and female (open bars) *Chorthippus parallelus*. Bars represent means \pm SE and numbers above bars give number of replicates.

on four very different diets from different habitats of the species. While some plant species were part of all food plant mixtures, several plant species only occurred in one or a few of the mixtures. Grasshoppers always included several plant species in their diet, but consumption of these species was different in the different treatments and hence depended on the availability of other food plants. Grasshopper diet selection

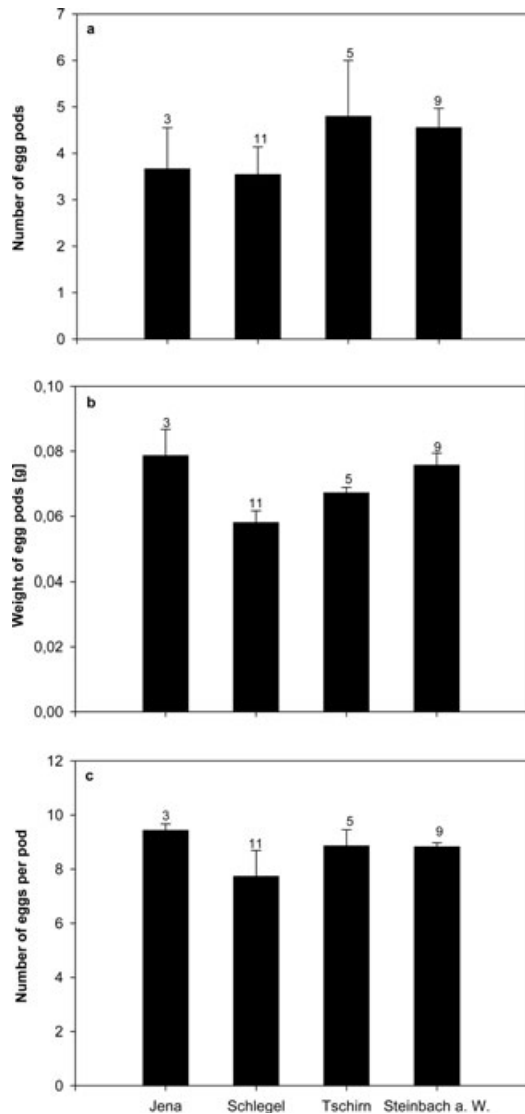


Fig. 6. Effects of food plant mixtures on (a) number of egg pods laid, (b) fresh weight of egg pods, and (c) number of eggs per egg pod. Bars represent means \pm SE and numbers above bars give number of replicates.

also depended on grasshopper developmental stage and sex. These results emphasise that there is active food plant selection and dietary mixing in the generalist grasshopper *C. parallelus* that apparently complemented for any deficiencies in particular plant species.

The consumption of small amounts of forbs and high amounts of particular legumes in addition to grasses by *C. parallelus* is consistent with results from other studies (Specht *et al.*, 2008; Unsicker *et al.*, 2008), even though there is a lack of quantitative data from natural conditions (Bernays & Chapman, 1970a; Illich & Winding, 1989; Ingrisch & Köhler, 1998). *Chorthippus parallelus* is therefore a true generalist herbivore, consuming plants from more than one plant family. Legumes were, in fact, consumed throughout the ontogeny

at fairly constant rates (Fig. 1). In our study diet breadth tended to be broad in early instars, when almost all plant species were eaten, then decreased and was lowest in the third and fourth nymphal stage, while adult grasshoppers had a somewhat broader diet (Fig. A1). Preferences of *C. parallelus* grasshoppers for particular food plant species were distinct throughout the development, and while there were some differences between individuals within a treatment, the response to a particular food plant mixture was relatively similar. All food plants were provided *ad libitum* so it can be stated with confidence that there was active food selection behaviour. Importantly, grasshoppers tested all of the plant species, mainly during the nymphal phase of grasshopper development. While many of the herbs were consumed in very little amounts, their contribution to grasshopper fitness may have been non-negligible, but to understand the importance of including small amounts of particular herbs into the diet would require further experimentation.

Preferences for particular food plants have been shown in most generalist herbivores investigated, including grasshoppers, and many detailed studies have shown how herbivores compensate for deficiencies in, for example, nitrogen by increasing feeding rate or adjust feeding preferences (e.g. Yang & Joern, 1994; Bernays & Minkenberg, 1997; Miura & Ohsaki, 2004a, 2006; Berner *et al.*, 2005). In our experiment, feeding preferences differed between the food plant mixtures offered (Fig. 1, Fig. A1). These differences were more striking than they appear to be at first sight. For example, in the 'Schlegel' and partly the 'Tschirn' treatment, legume consumption accounted for more than 10% of the diet across all nymphal stages, whereas in the other mixtures legumes comprised well below 10%. One possible reason for this feeding pattern is the low number of grass species in the 'Schlegel' mixture which was about half the number of grass species in the other three food plant mixtures. There were also clear differences in preferences between the different grass species which could be due to either the physical parameters of these grass species (e.g. toughness or hairiness), as well as their secondary metabolites which may act as feeding deterrents. Feeding preferences of the grasshoppers strongly depended on the plant species mixture offered. In contrast to the differences among grasses, the two legume species *T. pratense* and *T. repens* were equally preferred. Feeding preferences also depended on sex and developmental stage (Figs 1–3). For example, in nymphal stage 4, females consumed more legumes than males. This could be due to a higher need for nitrogen at the life stage when female grasshoppers start to produce egg pods (e.g. Bernays & Simpson, 1990). Shifts in food plant preferences related to the grasshoppers' ontogeny have also been observed in another study on *C. parallelus* by Unsicker *et al.* (2008). Based on results from our study, it is evident that *C. parallelus* does not randomly feed on the offered plant species but rather actively performs dietary mixing, which is influenced by both grasshopper ontogeny and sex. With respect to this feeding behaviour, the urgent question arises as to whether dietary mixing in *C. parallelus* is primarily driven by the grasshoppers' need to maintain its nutritional balance (nutrient complementation hypothesis: Pulliam, 1975; Rapport, 1980) or

by the necessity to dilute plant allelochemicals that act as toxins on the grasshoppers (toxin dilution hypothesis: Freeland & Janzen, 1974). As we did not investigate the secondary metabolites and the available nutrients of the plant species we offered to the grasshoppers in this study, we can only speculate on the mechanisms underlying *C. parallelus* feeding preference. Studies in the future should address these issues.

In this study, we did not analyse the nutritional quality of the different plant species, but CN analyses (J. Specht, S. Unsicker and W. Weisser, unpublished) indicate that the C:N ratio of grasses of the unfertilised meadows in the study area ranges between 15 and 17 with little differences between different meadows. However, C:N ratio is but one indicator of plant nutritional quality for grasshoppers and generally, N-content is a poor descriptor for plant defences (Schoonhoven *et al.*, 2005). Since investigating the physiological details of choosing particular plant species was beyond the scope of the present study, it is possible that there were variations among meadows in the nutritional quality of the plant species. The fact that grasshoppers showed clear preferences for several plant species in each mixture suggest that grasshoppers chose a balancing feeding strategy.

There were differences in the efficiency of conversion of ingested food to body substance (ECI) and the efficiency with which digested food is converted to body substance (ECD) between the food plant mixtures (Fig. 4b,c). Waldbauer (1968) argued that differences in ECI are related to differences in the digestibility of the diet. Low ECD values are often coupled with high AD values (Waldbauer, 1968), which was also true for our experiment, although differences in AD were not significant (Fig. 4). In a study by Miura and Ohsaki (2006), ECD was higher in individuals that fed on qualitatively better plant species and food plant mixtures. In the case of our study, for both males and females, ECI and ECD were highest for the 'Steinbach a. W.' mixture and lowest for the 'Jena' mixture, especially for females. This corresponded to the pattern in total consumption, which was highest in the 'Jena' mixture and lowest in the 'Steinbach a. W.' mixture, suggesting that food in the 'Jena' mixture was of a lower quality and had to be consumed in greater quantities. Berner *et al.* (2005) also observed that the grasshopper *Omocestus viridulus* consumed almost twice as much biomass in a low-quality (in this case low nitrogen) food than in a high quality (high N) food. The emphasis in our study was on recording individual feeding behaviour, and we therefore did not sacrifice individuals during the study. Hence, we had to calculate ECI and ECD using dry weight after death (for females at the end of the experiment and for males after their copulation with females). Thus, the results on ECI and ECD have to be taken with some caution and should only be seen as an indication of differences in the diets.

Despite the differences in plant species composition and food consumption, there were no differences in fitness measures between the food plant mixtures except for egg pod weight. This was, however, not related to egg numbers in the egg pods. Survival of the grasshopper individuals was high in all mixtures. Identical survival on different diets is consistent with the results of Berner *et al.* (2005) for *O. viridulus* feeding

on plants of high or low quality. Other feeding experiments with generalist insect herbivores, including grasshoppers using artificial diets deficient in nitrogen or other nutrients, have often found fitness differences among diets e.g. for locusts (Joern & Behmer, 1997; Raubenheimer & Simpson, 2003) or caterpillars (Fischer & Fiedler, 2000; Lee *et al.*, 2002), although there are many examples of compensatory feeding (Simpson & Raubenheimer, 2000). We investigated a number of different fitness measures over the lifetime of the grasshoppers including egg pod production, and in the selection of meadows of our study we deliberately chose plant communities that were very different from one another. Our results show that in the generalist *C. parallelus*, differences in plant species composition alone among the natural habitats may not be sufficient to bring about differences in fitness measures. Although our study was not designed for testing local adaptation of grasshoppers to their habitats, the results also suggest that the grasshoppers caught at the 'Jena' site were not locally adapted to their food plants.

Unsicker *et al.* (2008) found that increasing the number of plant species in the diet of *C. parallelus* strongly increased grasshopper fitness, yet the highest number offered in their experiments was eight, lower than the lowest number of plant species in the present experiment, suggesting that compensatory feeding is possible as soon as there is sufficient choice among plant species. Unsicker *et al.* (2008) also showed that different mixtures of three plant species differed strongly in their effect on grasshopper survival and fecundity, emphasising the different suitability of different plant species for grasshopper development. In this experiment, grasshopper feeding on particular species also depended on the composition of the food plant mixture. On the other hand, Unsicker *et al.* (2009) found for the same study region that the fecundity of adult *C. parallelus* females from different meadows was positively correlated to plant species richness in the sites. This was true for both total species richness and species richness based only on grasses and legumes. In light of the current results, there are two possibilities to explain these results. First, there are likely to be factors other than diet breadth that correlate with high plant species richness in the field. While Unsicker *et al.* (2009) found that variables such as plant community biomass, solar radiation, or leaf area index did not explain the differences in fitness parameters, there may be other unmeasured variables, e.g. pressure by natural enemies or other microclimatic parameters that co-vary with plant species richness. Another possibility is that the feeding behaviour of grasshoppers in the field (as in Unsicker *et al.*, 2009) differs from their feeding behaviour under *ad libitum* conditions as in the present study. In our experiment all plant species were provided in sufficient amounts to make sure that they were not depleted during the 4-day interval between cage inspections, and the physical distances between the different plant species were very small so that it was possible for grasshoppers to find and feed on a particular plant species. This differs greatly from the situation in a meadow where plants are not homogeneously distributed, and where opportunity costs of searching for a particular species may occur. That grasshoppers including *C. parallelus* also show preferences for

particular host plants in the field has been shown by several studies (e.g. Bernays & Chapman, 1970a). In our study, there was—not unexpectedly—no correlation between the relative frequency in which a particular plant species occurred in the ‘Jena’ or any of the other meadows, and the fraction of this species in the respective diet, also showing that food plant preferences of *C. parallelus* have not evolved to mirror the plant species composition of the grasshoppers’ habitat. Field studies of diet selection behaviour of grasshoppers in different meadows would therefore be important, to investigate whether preference as measured in the laboratory matches preferences in the field. Unfortunately, analysing feeding preferences of grasshoppers in the field still requires extensive observations or gut dissections, both of which are very time-consuming. Most studies of grasshopper feeding behaviour have therefore focused either on qualitative analyses or concentrated on particular plant species or grasshopper developmental stages (e.g. Bernays & Chapman, 1970a; Miura & Ohsaki, 2004b). With the fast progress in the development of molecular methods that can identify plant species from fragments in the gut or faeces of herbivores (Valentini *et al.*, 2009a,b), comparative and quantitative diet selection analyses over the ontogeny of individuals in different habitats in the field will be facilitated. Our results emphasise, however, that when given the choice, *C. parallelus* mixes its diet in a way that results in similar fitness in the different plant communities, so it is not plant quality *per se* that brings about the differences in grasshopper fitness among habitats.

To summarise, our experiment shows that the generalist herbivore *C. parallelus* consumes a broad range of plant species but shows distinctive preferences that depend on the food plant mixture available. These preferences are also sex- and instar-specific, and the diet selection behaviour results, at least under conditions of unlimited access to plants, in equal fitness when offered food plant mixtures from several habitats in which the grasshopper occurs. This suggests that these generalist herbivores are indeed able to cope very well with the diet available in their habitats. The results also emphasise that any differences in grasshopper fitness among different habitats may not only result from differences in the quality of the food plants present in the different habitats or different abiotic conditions, but also from constraints on diet selection behaviour, for example due to the need of avoiding predation (Lima & Dill, 1989; Schmitz, 2003), which needs to be investigated in more detail.

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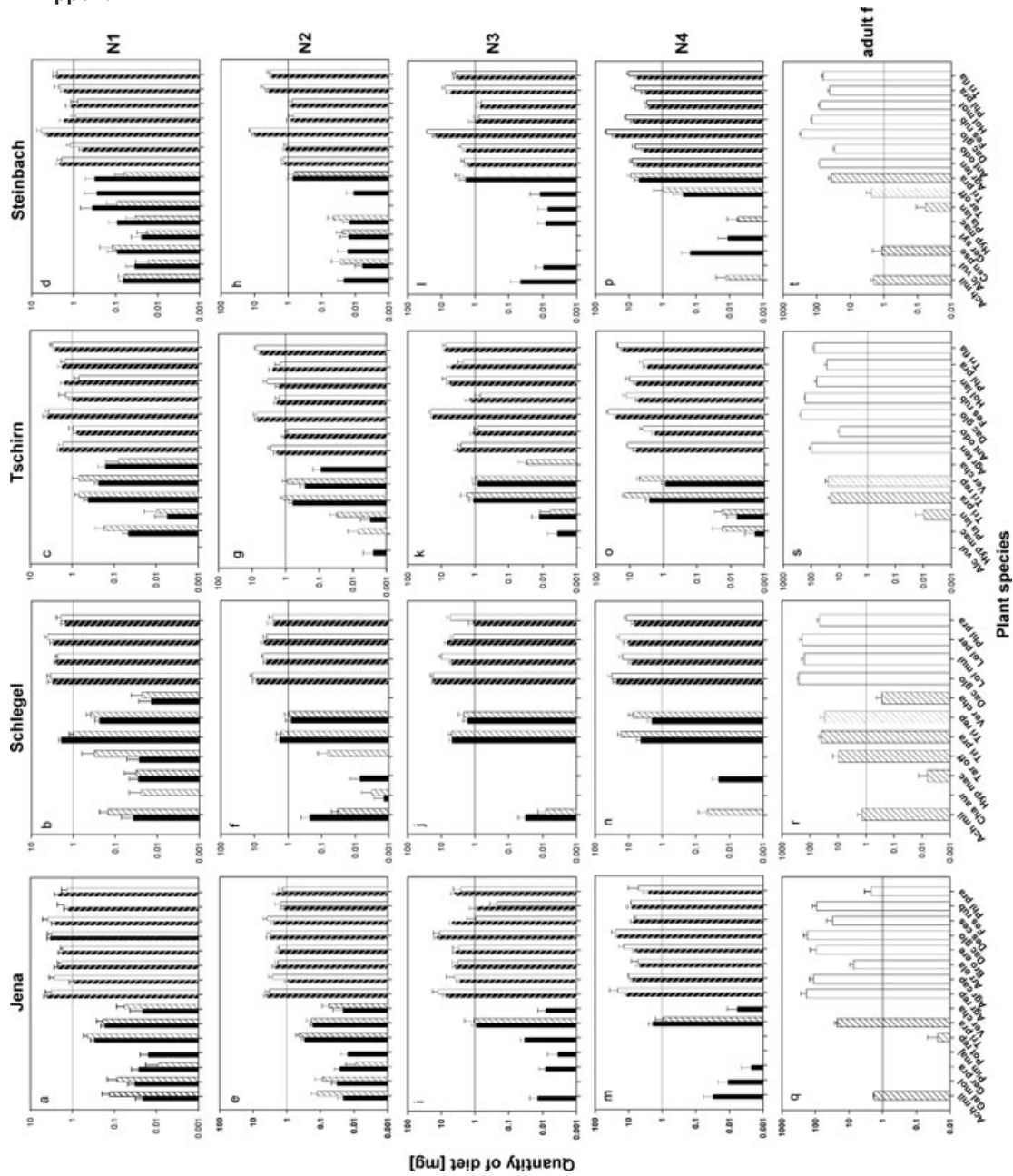
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Appendix



Appendix 1. Food consumption of grasshoppers in the experiment by grasshopper sex, instar, treatment, and plant species. As males were sacrificed after maturity, consumption of adults was only measured in females. Colour and pattern of bar indicate grasshopper sex and plant functional group: Full bars = male feeding on forbs/legumes, full bars hatched: male feeding on grasses; open hatched bars = female feeding on forbs/legumes, open bars: female feeding on grasses. (a) 'Jena', (b) 'Schlegel', (c) 'Tschirn', (d) 'Steinbach'. Bars represent means \pm SE. Note that the y-axis is in logarithmic scale.