

Grasshoppers cope with low host plant quality by compensatory feeding and food selection: N limitation challenged

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The effect of low host plant nitrogen (N) content on herbivore performance has rarely been studied together with the herbivore's feeding behaviour. We explored this relationship with juvenile *Omocestus viridulus* (Orthoptera: Acrididae) grasshoppers using fertilized and unfertilized host grasses. Due to lower growth rates, grasshoppers reared on N-poor grasses exhibited slightly prolonged development and smaller adult size, while mortality was similar among the fertilizer treatments. This was found both in the laboratory and in outdoor cages under natural climatic conditions. A parallel analysis of feeding behaviour revealed that the grasshoppers counterbalance N shortage by compensatory feeding, and are capable of selectively feeding among grasses of contrasting nutritional quality when given a choice. This indicates a striking ability of *O. viridulus* to regulate nutrient intake in the face of imbalanced food sources. Although the species exploits a relatively very poor autotroph nutrient base in the wild, as underpinned by N analysis of natural host grasses and grasshopper tissue, our data suggest that natural food quality imposes no relevant constraint on the herbivore's performance. Our study thus challenges the importance of simple plant-mediated control of herbivore populations, such as N limitation, but supports the view that herbivores balance their intake of N and energy.

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By virtue of a large mismatch in tissue nitrogen contents between terrestrial autotrophs and consumers (Elser et al. 2000), nitrogen is often considered the key nutrient required by herbivorous arthropods (Mattson 1980, Bernays and Chapman 1994, Schoonhoven et al. 1998). This has led to the nitrogen limitation concept, which holds that the natural nitrogen (N) content of food plant tissues generally impairs consumer performance (White 1993). Although in some cases specific biochemicals rather than bulk N seem to constrain herbivores (Behmer and Joern 1993, Anderson et al. 2004), consumer survival, growth and/or fecundity often respond positively to increased N (or protein) availability

(Smith and Northcott 1951, Slansky and Feeny 1977, Lincoln et al. 1982, Ravenscroft 1994, Joern and Behmer 1997, Wheeler and Halpern 1999). The notion of N limitation thus rests on substantial empirical evidence and has lately been extended to the level of carnivorous arthropods (Denno and Fagan 2003). However, studies with crustaceans (Cruz-Rivera and Hay 2000) and bugs (Di Giulio and Edwards 2003) recently demonstrated that some herbivores are able to overcome N shortage while close relatives are indeed strongly N limited. This suggests that predictions based on N limitation may not account for the diversity of herbivore responses to low N resources.

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Over the recent years, an alternative to the strictly one-dimensional view of N limitation has emerged, the nutrient balance (or “geometric”) framework (Raubenheimer and Simpson 1999, 2004, Simpson and Raubenheimer 2000). This concept emphasizes the simultaneous regulation of the intake of nitrogenous nutrients and carbohydrate-derived energy by herbivores. Experimental work on nutrient balance, focused primarily on insect herbivores (but see Mayntz et al. 2005) and artificial diets, has shown that both protein and carbohydrate need to be ingested in a specific amount over a given period of time to sustain optimal growth and development. This nutritional target can be effectively stabilized over a wide range of food protein and carbohydrate compositions due to the interplay of several behavioural mechanisms. These include selection among unbalanced but complementary resources (Waldbauer and Friedman 1991, Chambers et al. 1995, Behmer et al. 2001, Lee et al. 2002), compensatory feeding by adjusting the total amount of food ingested (Abisgold and Simpson 1987, Raubenheimer and Simpson 1993), and post-ingestive regulation (Zanotto et al. 1993, 1997). Central to studies of nutritional balancing is the premise that animals must trade off the costs of overeating excess nutrients against undereating those in deficit in the diet, as recently modelled and tested by Simpson et al. (2004).

The objective of the present study is to explore the feeding behaviour and performance of *Omocestus viridulus* (L.) grasshoppers in relation to natural host grasses of contrasting N contents, and to evaluate the results against predictions provided by the nitrogen limitation hypothesis as well as its alternative, the nutrient balance framework. For several reasons, the grasshopper species chosen is particularly suited for this task. First, *O. viridulus* feeds exclusively on grasses. Among insects consuming plant foliage, strict grass-feeders exploit a particularly poor nutritional environment, due to generally lower leaf N contents in grasses compared to herbs (Bernays and Barbehenn 1987). Grass-chewers are found mainly in the major insect orders Lepidoptera, Coleoptera and Orthoptera. Orthopterans, including grasshoppers, display substantially elevated tissue N contents compared to the other, more derived orders (Fagan et al. 2002). Grass-feeding grasshoppers thus represent excellent models to investigate whether organisms with relatively high N demands and naturally confined to poor food sources are N limited, and if and how they balance their nutrient intake. Furthermore, plant secondary compounds, which may confound interpretations based on nutrients, are of little importance in grasses (Bernays and Barbehenn 1987). In addition, the species is a representative of a large group of grass-feeders that often constitute the top arthropod grazers in grassland ecosystems (Blumer and Diemer 1996, Ingrisch and Köhler 1998). Consequently, the significance of bottom-up (plant-mediated) con-

straints to this grazer is of high interest to food web theory.

Specifically, we examined the relative impact of high and low N food on juvenile survival, growth and development. This was done both in the laboratory and under natural outdoor conditions using cages. Given that natural conditions can strongly influence insect responses to food quality (Hunter and McNeil 1997), and that hardly any experimental work dealing with nutrient limitation has been conducted outside the laboratory (Ravenscroft 1994), the outdoor replication clearly increased the scope of our study. We focused on juvenile stages, as high N requirements for tissue growth render juvenile arthropods particularly susceptible to inadequate food quality (Scriber and Slansky 1981, White 1993, Zalucki et al. 2002). Separate experiments were carried out to reveal possible compensation of low food N through adjustment of consumption rates, and to establish if the animals are capable of selecting among food sources of contrasting nutritional quality. Although such behavioural responses have been reported for several arthropods, mostly using artificial diets (Simpson and Simpson 1990; but see Wright et al. 2000), they have rarely been combined with performance experiments (Slansky and Feeny 1977, Cruz-Rivera and Hay 2000, Raubenheimer and Simpson 2004, Simpson et al. 2004). Hence, their ecological significance mostly remains unexplored. Finally, we determined N contents of grasshopper tissue and grass samples from the field. This allowed quantifying the discrepancy in tissue N between resource and consumer, and evaluating experimental N levels against those naturally occurring.

Material and methods

Host grass sampling in the field

In order to investigate naturally occurring host N contents, a total of 22 samples from 13 grass species (including one sedge) were taken in 2002 and 2003. We sampled six grassland sites in northeastern Switzerland where *O. viridulus* occurs. Only grass species with relatively high dominance at a site were considered, and all species had been observed to be readily consumed by *O. viridulus* in the wild in a preliminary foraging study. At each site, grass sampling took place during the end of grasshopper juvenile development (June/July), thus taking into account the herbivore's phenology. For a sample, we cut 20–40 g fresh weight of leaf blades from several individual plants.

Study organism

All experiments were conducted with the first offspring generation of *Omocestus viridulus* (Acrididae: Gompho-

cerinae), a grass-feeding grasshopper widespread in central Europe. It displays one generation a year and has four nymphal (juvenile) stages. Males exhibit slightly faster nymphal development and smaller body size compared to females. We caught ten males and females from each of three locations in northeastern Switzerland in July 2003. The animals were kept in groups in cages in the greenhouse and allowed to reproduce until death. A field-cut grass mixture served as food. Egg pods were put individually in plastic tubes containing moist vermiculite. Subsequently, they were incubated for one month in a climate chamber set to 25°C, followed by four months at 5°C for diapause. Further incubation with 14 h days at 27°C (night 8°C) for 2–3 weeks yielded hatchlings for experiments. To obtain experimental grasshoppers in the last (4th) nymphal stage, hatchlings were transferred to 32°C (fluctuating diurnally) and raised in groups in cages on a grass mixture from the greenhouse.

Experimental host grass

The four Poaceae *Dactylis glomerata*, *Festuca pratensis*, *Holcus lanatus* and *Trisetum flavescens* were grown from seeds in the greenhouse. A mixture of the four species, all of which belong to the grasshopper's natural diet, was utilized in all trials to mimic natural conditions. Each species was sown separately in pots of 16 cm diameter and 13 cm height. As substrate we used a sand-soil mixture (volume ratio 11:6). Four weeks after germination the grass was assigned one of two treatments: the high N treatment involved an application of 0.1 l of a standard fertilizer solution (Wuxal, 0.4%; Maag Agro, Dielsdorf, Switzerland), corresponding to 40 mg N, every five days. The low N treatment pots received no N fertilizer, but 0.3 l of a modified Hoagland solution was applied weekly. This solution contained all plant nutrients in balanced proportion except for N and was used to make sure that N was the only element in short supply. Furthermore, all pots were maintained moist by watering every other day. The grasses were grown in five temporal series separated by one week and used 8–9 weeks after germination. For a later N analysis, we drew one sample per species and treatment from three series.

Grasshopper performance

Juvenile development on high and low N grass was studied in a climate chamber set to a 16 h light phase at 32°C. We chose this temperature taking into account the high temperature demands of grasshoppers (Ingrisch and Köhler 1998), which are met in the field by absorption of solar radiation (Begon 1983). Night temperature was 10°C and relative humidity 40% throughout. Fresh hatchlings from one population were randomly assigned to an N treatment and indi-

vidually transferred to plastic containers of 19 cm height and 8 cm diameter. As food, two leaf blades per grass species of the corresponding treatment were cut from the greenhouse pots, offered in small water-filled glass vials, and replaced every third day. Twice daily, we examined each container, noted new moults, and collected nymphal skins until the grasshoppers reached the adult stage. Hind femur length (henceforth referred to as body size) of nymphal skins and adults was measured using a stereomicroscope. The linear regression slope of body size against development time across the five stages (nymphal 1–4, adult) served as an estimate of individual growth rate. Family (=clutch) means of growth rate, adult body size and development time were analysed as general linear models (GLM) with sex and N treatment as fixed factors. Data from 9–13 families per sex by treatment combination were available (39 in total). To analyse individual survival to adulthood, we chose Fisher's exact test because expected values in two cells were slightly below five. Here, sample size was 38 and 39 animals for the high and low N treatment, respectively. SPSS 11.5 was used for statistics throughout.

To study juvenile performance under natural climatic conditions, we established grass of contrasting N supply in a similar way as outlined above. Before the experiment started, the soil substrate in each grass pot was cut into quarters. Within each N treatment, soil quarters from each of the four grass species were reassembled in new pots, yielding high and low N grass mixtures. Subsequently, we equipped every pot with a steel frame of 45 cm height and 15 cm diameter that was coated with a nylon stocking. To launch the experiment, six grasshopper hatchlings from one population were introduced in each of these resulting cages. After this, we placed the cages outdoors in an open area at the Swiss Federal Research Station for Agroecology and Agriculture. This was done between May 10 and 14 2004, corresponding to the species' natural hatching period (Bernier et al. 2004). Animals of both sexes were used, and their allocation was random. The setup comprised six high N and seven low N cages. During the whole experimental period, the high N pots received 0.1 l of the standard fertilizer solution every 6–8 days. The low N treatment involved an application of 0.1 l of the fertilizer solution diluted to 1/3 with the modified Hoagland solution every ten days. In addition, the pots were watered if required. Some nutrient loss owing to rain washout certainly occurred but was not controlled for. For later N analysis, two grass samples were drawn per treatment both in the beginning and after ca 2/3 of the experimental period. In order to standardize the microclimate in the cages, we cut back the grass to 25 cm height weekly. As indicated by hourly data logger ("StowAway TidbiT", Onset Computer Corporation, Bourne, MA, USA) measurements over several weeks within and outside two additional cages (without grass-

hoppers), cage temperatures did not systematically deviate from ambient temperatures. For each individual, we determined total nymphal development time with a resolution of 3–4 d and adult body size. Cage means of development time and size for each sex were analysed using GLM, with treatment and sex as fixed factors. Survival expressed as the raw number of resulting adults per cage was analysed with a two-sample t-test.

Feeding behaviour

Food consumption on high and low N hosts was investigated in the laboratory using females from one population on day two of their final nymphal stage. We used the same chamber conditions and containers as above. The animals were starved for 3 h prior to the experiment to empty their guts. Each grasshopper was offered eight freshly cut leaf blades, two per grass species, of either treatment (non-choice setting). This was enough plant material so that the animals could not deplete it completely during the trial, and its fresh weight was determined immediately after cutting (all eight blades pooled). The grasshoppers were then allowed to feed for an entire light phase, subsequently starved for 3 h, frozen and dried at 45°C for 72 h. Individual food consumption in terms of dry weight was calculated gravimetrically. For this, we converted initial grass fresh weight to dry weight using a multiply determined constant for each N treatment, and subtracted the dry weight of the grass leftovers. Consumption on a fresh weight basis could be estimated directly. We analysed consumed dry and fresh matter using GLM with treatment as fixed factor and nymphal dry mass as continuous covariate (ANCOVA approach; Raubenheimer and Simpson 1994, Raubenheimer 1995). Sample size was 22 and 23 for the low and high N treatment, respectively. Two random samples, each of 20 dried nymphs, were retained for analysis of grasshopper tissue N content.

The occurrence of food selection behaviour was investigated with nymphal females (age as above) under the laboratory conditions already described. Containers of 11 cm in diameter and 15.5 cm high were utilized. They were provided with two water-filled glass vials, each containing 12 blades (three per species) of high or low N grass, resulting in a choice setting. In the middle of the containers, we inserted a vertical cardboard wall of 10 cm height to separate the vials. This allowed attributing grass leftovers to the corresponding N treatment, but did not impede grasshopper movement. One animal was introduced per container and left to feed for 44 h (28 h light phase). For each of the 16 individuals studied, we gravimetrically assessed the dry mass of high and low N grass consumed. A paired-samples t-test was used for analysis.

Nitrogen analysis

Plant material destined for N analysis was oven-dried for 72 h at 50°C. All samples were ground in a sample mill equipped with a 0.5 mm screen (Cyclotec 1093, Foss Tecator, Sweden). N content was determined by combustion at 900°C in an elemental analyzer (vario MAX CN, Elementar, Hanau, Germany). Some samples analysed in duplicate showed that measurement error was negligible (mean 1.6%, maximum 3.9%).

Results

Nitrogen contents

In grass samples from the field, tissue N on a dry weight basis ranged from 1.76% to 3.7% with a median of 2.19% (Fig. 1). There was considerable within-plant species variation across sites: *Dactylis glomerata* samples, for instance, differed by more than 40% (2.2%–3.2% N). Such difference partly arises from fertilization, as the three samples with the highest N content stemmed from a manured meadow.

Fertilizer application caused a consistent increase of N content in grass leaves used in the laboratory trials (Fig. 2). Averaged across the four grass species, N content in fertilized grass was 2.7-fold higher than in unfertilized grass (4.3% vs 1.6% N respectively). In the outdoor cages, fertilization increased leaf N roughly twofold from 1.2% to 2.5% N (Fig. 2). Finally, N accounted for 11.3% of grasshopper tissue dry weight.

Grasshopper performance

In the laboratory, grasshopper survival to adulthood was somewhat higher on unfertilized than on fertilized grass (37 of 39 [95%] vs 32 of 38 [84%], respectively). The difference, however, was non-significant ($p=0.154$). Juvenile mortality occurred mainly during the first

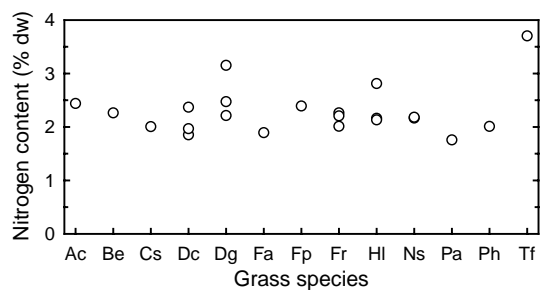


Fig. 1. Nitrogen contents of host grasses collected in the field. The species are from left to right *Agrostis capillaris*, *Bromus erectus*, *Carex sempervirens* (Cyperaceae), *Deschampsia caespitosa*, *Dactylis glomerata*, *Festuca arundinacea*, *F. pratensis*, *F. rubra*, *Holcus lanatus*, *Nardus stricta*, *Poa alpina*, *Phleum alpinum*, *Trisetum flavescens*.

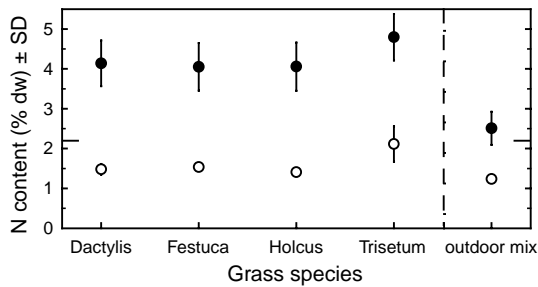


Fig. 2. Nitrogen contents of unfertilized (○) and fertilized (●) experimental grasses used in the laboratory (four species, left) and outdoors (mixture, right). The horizontal bar in the scale indicates the median N content of field samples (from Fig. 1). Some error bars are hidden by their symbol.

nymphal stage. Growth rates of animals developing on low N grass were reduced as compared to those on high N food ($F_{1,35}=62.7$, $p<0.001$; Fig. 3). Consequently, juvenile development of grasshoppers fed low N grass was prolonged by 2.4 d (13%) on average ($F_{1,35}=45.5$, $p<0.001$). Moreover, these animals reached 5% smaller adult body size ($F_{1,35}=22.8$, $p<0.001$). The sexes responded similarly, as no sex by treatment interaction was significant (all $p>0.12$).

The outdoor cage experiment yielded very similar results. Juvenile survival did not significantly differ between the N treatments ($t_{11} = -1.125$, $p=0.285$) and was close to the laboratory survival rates (high N: 28 of 36 [81%], low N: 37 of 42 [88%]). Grasshoppers in unfertilized cages reached adulthood 3.6 d (7%) later on average ($F_{1,21}=5.566$, $p=0.028$) and attained ca 4% smaller size ($F_{1,21}=20.5$, $p<0.001$), with similar responses in the sexes (interactions $p>0.26$). We consider these differences marginal.

Feeding behaviour

On a dry weight basis, the overall mean food consumption of grasshoppers feeding on low N grass was 82% higher than on fertilized grass ($F_{1,42}=48.8$, $p<0.001$; Fig. 4). In terms of fresh weight, the consumption was

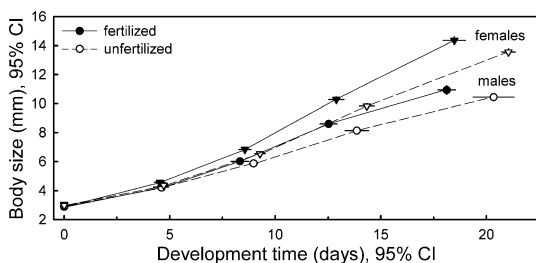


Fig. 3. Growth from the first nymphal stage to adult of male and female grasshoppers reared on unfertilized and fertilized grass in the laboratory, based on family means. Some error bars are hidden by the data point.

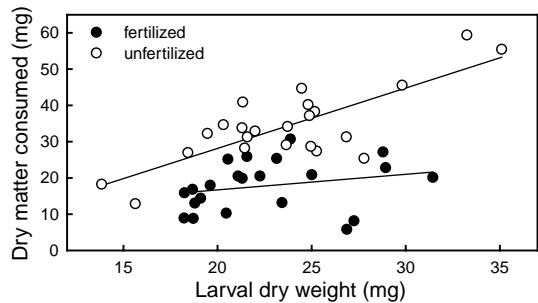


Fig. 4. Dry matter consumption over a 16 h trial period by female *O. viridulus* 4th stage nymphs feeding on low or high N grass. Consumption, adjusted for nymphal weight, is significantly higher on unfertilized grass.

still 40% higher ($F_{1,42}=15.1$, $p<0.001$). This indicates that grasshoppers compensated for lower nutritional quality by eating more.

The food selection experiment revealed a striking preference for fertilized grass (paired $t_{15} = -8.99$, $p<0.001$; Fig. 5). When there was a choice, individual grasshoppers consumed an average of 39 mg high N grass (dry weight) during the test period, as opposed to only 8 mg low N grass.

Discussion

Our analyses revealed that *O. viridulus*' natural host grasses contain around 2% tissue N, a relatively low value typical of grasses (Bernays and Barbehenn 1987). Further, qualitative inspection of the database compiled by Fagan et al. (2002) suggests that with 11.3% *O. viridulus* does not differ in tissue N content from other grasshopper species that generally exhibit N demands particularly high for herbivores. Along the lines of N limitation, we therefore expected high

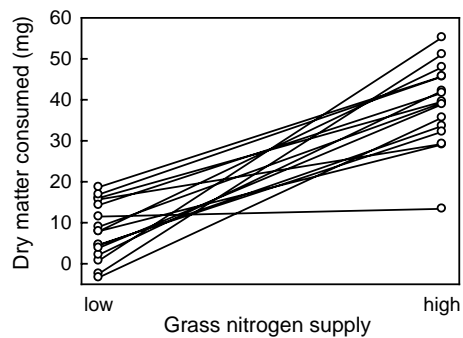


Fig. 5. Dry matter consumption over a 28 h period by female *O. viridulus* nymphs offered unfertilized and fertilized grasses simultaneously. Each line represents one individual. A clear preference for high N grass is evident. The two negative values in the data set point to some estimation error associated with the gravimetric method applied.

susceptibility of juvenile performance to experimentally modified host plant quality.

Surprisingly, survival in the laboratory of *O. viridulus* nymphs feeding on low N grass was not lower than on fertilized grass despite a nearly threefold difference in grass tissue N content. Grasshoppers attained almost the same body sizes on both N treatments, although reduced growth rates on low N grass prolonged development to some extent. Grasshopper survival in the wild is known to be severely reduced by wet weather periods (Joern and Gaines 1990, Ingrisch and Köhler 1998), at least partly due to fungal infection (Streett and McGuire 1990). Whether these sources of mortality interact with food quality has to our knowledge never been investigated. We observed no such effect, as the outdoor experiment yielded results highly consistent with those from the benign laboratory environment. This finding is striking for two reasons. First, the low N grass employed outdoors was of extremely poor quality (1.2% N), containing only about half as much N as grass from the field, whereas the fertilized treatment (2.5%) matched natural levels. Secondly, rainfall was roughly 20% above average during the trial period (MeteoSwiss, pers. comm.), indicating rather unfavourable climatic conditions. The weak performance differences in response to the tremendous difference in food plant N, exhibited under strikingly different rearing environments, leads us to conclude that naturally occurring grass N contents impose few constraints upon juvenile survival, growth and development of *O. viridulus*. Our results disagree with the simplistic claim that natural autotroph tissue N inherently limits herbivore performance (Mattson 1980, White 1993).

In several respects, however, our data are consistent with the view that herbivorous insects balance their nutrient budget. One means to stabilize nutrient intake, and thus growth and developmental performance, is through compensatory feeding. Indeed, dry weight food consumption of *O. viridulus* nymphs exposed to low N grass was almost twice as high as on fertilized grass. However, differing water contents of food plants may result in differential dry weight consumption even if fresh weight intake is similar (Wheeler and Halpern 1999), and thus may lead to the erroneous inference of compensatory feeding. The fact that our experiments also revealed a 40% increase of fresh matter consumption on low quality grass indicates that elevated food intake is a real behavioural response of *O. viridulus* to low grass N content. Similar intake adjustment in response to nutrient dilution or imbalance has been documented for crustaceans (Cruz-Rivera and Hay 2000), slugs (Rueda et al. 1991), and other insects (Slansky and Feeny 1977, Karowe and Martin 1989, Raubenheimer and Simpson 1993, Stockhoff 1993, Obermaier and Zwölfer 1999, Jones and Raubenheimer 2001). This suggests that compensation is a common

phenomenon in invertebrates. To compensate for protein dilution of artificial diets, the grasshoppers *Locusta migratoria* (Simpson and Abisgold 1985) and *Melanoplus differentialis* (Yang and Joern 1994) increased feeding frequency while meal size remained constant. Although we did not observe feeding behaviour directly, it is very probable that the same behavioural response holds for *O. viridulus* on the natural hosts used.

Two further issues arising from the compensation experiment deserve discussion. On the one hand, it is evident that compensation was not fully successful in the grasshoppers fed low N grass, as these animals exhibited somewhat prolonged nymphal development and slightly decreased body size. Similar developmental responses when exposed to highly N deficient artificial foods have been reported previously for locusts (Raubenheimer and Simpson 1993) and caterpillars (Lee et al. 2002). Most probably, the degree of N dilution in the low N grass exceeded the digestive capacity of the grasshoppers; hence, nitrogen could not be derived from the food at a rate needed to maintain nutritional homeostasis and thus optimal growth. On the other hand, we can assume that growth occurred at a close to optimal rate in grasshoppers fed high N grass. Nevertheless, our data suggest that here mortality was higher compared to the low N treatment, although the trend was not significant. A possible explanation accounting for this pattern lies in the fact that compensatory feeding implies a tradeoff in that one nutrient is always ingested in excess of the physiological requirements unless the nutrients are perfectly balanced (Zanotto et al. 1993, 1997, Simpson et al. 2004). Using the conversion constant of 6.25 to estimate crude protein from total N (Allen 1989), we obtain an average protein content of 27% for the high N grass. Although we did not measure carbohydrate levels, it is highly probable that our high N grass was N-biased relative to the grasshopper's nutritional needs, since *L. migratoria* grasshopper nymphs performed best on artificial food containing only 19% dw protein and 23% carbohydrate (Chambers et al. 1995). *O. viridulus* nymphs fed fertilized grass thus ingested N in excess of physiological demands to satisfy their carbohydrate requirements, which likely entailed N loading and a survival cost. This view is supported by the finding that high growth rates associated with high N supply came at the expense of juvenile survival in butterflies (Fischer and Fiedler 2000) and locusts (Raubenheimer and Simpson 2004). However, further detailed experiments are needed to clarify these relationships in our species, and to exclude alternative explanations such as an intrinsic tradeoff between growth rate and survival (Arendt 1997).

The above results show that compensatory feeding enables grasshoppers reaching or approaching their nutrient target when exposed to highly unbalanced natural food. However, compensation not only implies

surplus consumption and processing of non-limiting food components, but also greater feeding effort that can increase predation risk (Loader and Damman 1991, Werner and Anholt 1993, Eklöv and Halvarsson 2000). Hence, given an appropriate choice of foods, it is generally a better response to stabilize nutrient intake by food selection. Indeed, some experiments have demonstrated the ability of herbivores to achieve an optimal nutrient balance by switching between unbalanced but complementary synthetic foods (Chambers et al. 1995, Behmer et al. 2001, Lee et al. 2002). As argued above, our high N grass was N-biased to some degree relative to the grasshoppers' nutritional needs. In contrast, the low N grass was strikingly N-deficient but likely provided an ample carbohydrate source, given that sugar and starch contents of grass leaves increase with decreasing plant N supply (Bernays and Chapman 1994, Marschner 1999). If the grasshoppers optimized N and carbohydrate intake simultaneously, we would therefore expect the animals to feed predominantly on high N grass, but complement their diet with energy-rich low N grass. The results from our food selection experiment are entirely consistent with this nutrient balancing view. However, did the animals really select for nitrogen and carbohydrates, or is the observed selection pattern due to other plant characters changing with N supply, such as water content or leaf toughness? In general, turgescence of plant tissues exceed grasshopper water demands (Bernays and Barbehenn 1987). Furthermore, *O. viridulus* did not discriminate against grasses like *Bromus erectus* or *Festuca arundinacea* in a preliminary choice trial. The leaves of these species proved particularly tough in a subsequent analysis (Berner, unpubl.), confirming that physical leaf properties are unlikely to influence grasshopper feeding behaviour (Bernays and Chapman 1970, Heidorn and Joern 1984, Chapman 1990). Therefore, we believe that our inference of food selection balancing the intake of key nutrients is valid.

The selection pattern observed raises the question about the proximate control of food choice behaviour in *O. viridulus*. Two different, mutually not exclusive mechanisms likely provide the answer. The first mechanism involves the modulation of taste receptor responsiveness by blood nutrient concentration. As shown for locusts (Simpson and Simpson 1990, Simpson et al. 1995, Cook et al. 2000), increasing levels of amino acids in the blood reduce the stimulatory responsiveness of the corresponding taste receptors on the mouthparts and legs. Hence, a protein-deficient individual (exhibiting a low blood amino acid level) will readily start feeding on a protein-rich food source. The same food, however, is less attractive and may not stimulate feeding when the animal's blood amino acid level is high. Since the same mechanism applies to carbohydrates, nutrient regulation based on the herbivore's nutritional status may cause selective feeding. On the other hand, a learned associa-

tion of food quality with an environmental cue via nutritional feedback may underlie the documented food selection (Simpson and White 1990, Waldbauer and Friedman 1991, Bernays 1995). Food selection based on associative learning in insects commonly involves olfactory stimuli, but visual cues are used as well (Raubenheimer and Tucker 1997). It is possible that the *O. viridulus* individuals learned the spatial location of the food sources rich in N and carbohydrate, as reported for locusts (Dukas and Bernays 2000).

Overall, the present study illustrates that natural food imposes no relevant nutritional constraint on the performance of *O. viridulus* grasshoppers. The species is capable of behaviourally stabilizing its nutrient intake over an impressive range of food qualities. Our study using real plants thus corroborates a body of work that has documented nutrient balancing in herbivores based on synthetic diets. However, our data challenge the general utility of simple bottom-up approaches to consumer population dynamics, such as N limitation.

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