

Anthony Joern · Spencer T. Behmer

Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae)

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Abstract Key demographic traits in insect herbivores (survival, growth, and egg production) are often responsive to variation in diet quality, especially to dietary nitrogen (N) levels. Soluble carbohydrates may also be limiting. Using defined diets under controlled laboratory conditions, we examined survival, growth, and egg production in response to a range of diet qualities in adult females of a grass-feeding grasshopper *Ageneotettix deorum* (Scudder). Diets varied factorially within naturally occurring ranges of total N (1–7%) and carbohydrate (4–27%) levels. N concentrations significantly impacted weight gain, egg production rate, the elapsed time until the first egg pod, and the time between the first and second egg pod. These responses were typically quadratic in nature with a maximum response near 4–5% total N. The rate of pod production rather than number of eggs per pod best explained changes in reproductive rate. Dietary carbohydrate levels seldom exerted a significant impact on demographic parameters except when interacting with N on survival, egg weight, and the period between egg pods. Clearly, factors that alter the availability of quality diet, especially total N levels, can contribute to demographic responses in *A. deorum*.

Key words Artificial diets · Demographic responses · Food quality · Nutrient limitation · Nutrient limitation hypothesis

Introduction

Under most natural conditions grasshoppers typically encounter abundant food plants. However, the nutri-

tional quality of food plants varies considerably within and among years, among sites, among plant species, and among tissues within a plant (McNeill and Southwood 1978; Mattson and Haack 1987; White 1993.) On a dry weight basis, average total nitrogen (N) in leaf material of naturally occurring grasses (g/g), for example, often ranges between 0.5–7%, while average total nonstructural carbohydrates (TNCs) may range from 5 to 10% in grasses to more than double this level in other plant taxa (A. Joern and S. Mole, unpublished data). Individual grass samples, however, often reached 30–40% TNCs (A. Joern and S. Mole, unpublished data). In addition, levels of dietary N and TNCs were not correlated in naturally occurring grasses for seven taxa sampled over 12 years. Consequently, it may be difficult for a grasshopper to repeatedly find and eat a quality meal, and the consequences of such variation in diet quality to growth, survival, and reproductive capability may be considerable.

Even under the best conditions, grasshoppers must often eat poor-quality food in relation to nutritional needs. For example, grasshoppers maintain high levels of total N in body tissues (Bernays 1982; Hinks et al. 1993), but typically eat food containing much lower N concentrations. To make up for the differences in concentration between body requirements and food levels, individuals must eat and convert a significant amount of plant material relative to body size. However, the restricted gut volume available for holding food, coupled with the associated digestive constraints, limits the amount of food that ultimately can be eaten and digested (Hinks et al. 1993; Yang and Joern 1994a, b), thus regulating the availability of resources to an individual. A grasshopper must allocate resources from this finite pool; a larger pool increases survival and enhances fecundity or growth (Roff 1992; Mole and Zera 1993). With a reduced pool of resources, a greater fraction must be allocated to metabolic processes that support survival. As a result, any change in food quality may routinely alter survival, development rate, growth rates, fecundity, or some combination of these, leading to a dynamic nutritional

A. Joern (✉)
School of Biological Sciences, University of Nebraska-Lincoln,
Lincoln, NE 68588-0118, USA

S.T. Behmer
Department of Entomology, University of Arizona,
Tucson, AZ 85722, USA

economy within individuals as demands from multiple biochemical and physiological functions compete for limited resources. Thus, limiting nutrients are differentially shunted to processes exhibiting the strongest need. More needs can be satisfied if nutritional resources are abundant compared with limiting situations. As a consequence, growth or reproduction will drop off or cease if the diet becomes inadequate (McCaffery 1975; Bernays 1982), and survival (reflecting maintenance capabilities) varies with diet quality as well (Smith and Northcott 1951; Joern and Gaines 1990). While selecting food resources, grasshoppers simultaneously obtain protein and energy in addition to other nutrients, including water (Dadd 1985). Depending on supply/demand relationships among competing physiological processes, needs for N (protein) and energy (carbohydrate) typically mold allocation schedules among competing physiological needs (Bloom et al. 1985; Hinks et al. 1993; Mole and Zera 1993).

Dynamic population responses by insect herbivores reflect integrated effects of multiple factors that affect basic demographic attributes (developmental rate, survival, and reproduction) (Cappuccino and Price 1995). In many cases, key demographic attributes vary in response to variation in food quality (Slansky 1982; White 1984; Mattson and Haack 1987; Joern and Gaines 1990), which in turn changes according to recent abiotic stresses on the plant. This issue has been presented most forcefully in the nutrient stress hypothesis (White 1993) and the more encompassing environmental stress hypothesis which includes dynamic responses by both plants and herbivores (Jones and Coleman 1992). For insect herbivores, available dietary N (protein and free amino acids) can potentially limit population processes (McNeill and Southwood 1978; Mattson 1980; White 1993). Some evidence suggests that naturally occurring protein levels in host plants can limit grasshoppers, although carbohydrate levels may be limiting as well (Dadd 1960, 1985; Joern and Gaines 1990). However, assessments of the relative impacts of dietary protein versus carbohydrates on demographic responses in insect herbivores are uncommon (Abisgold et al. 1994; Simpson et al. 1995).

In the present study, we evaluate the consequences of different quality diets (defined by dietary protein and carbohydrates) to adult female grasshoppers. We directly compare the consequences of varying dietary carbohydrates and protein (measured as N) levels in a factorial fashion to growth, survival, and egg production in the grass-feeding grasshopper, *Ageneotettix deorum* (Scudder). Since even free-living grasshoppers may be routinely forced to eat substandard foods under a variety of natural conditions, demographic responses dictated by growth, survival, and egg production may vary with different quality diets. In particular, we assess whether the natural intake rate of dietary carbohydrate and protein nutritionally limits (sensu White 1993) weight gain, survivorship, or egg production by *A. deorum*. These attributes can have great demographic impact.

Materials and methods

Grasshoppers

We examined adults of the strictly graminivorous grasshopper, *A. deorum*, an abundant grasshopper found throughout western US grasslands which typically feeds on grama grass species [*Bouteloua gracilis* (H.B.K.) Lag. ex Griffiths and *B. hirsuta* Lag.] (Joern 1979, 1985). *A. deorum* typically emerges in late May or early June with adults appearing in mid July. The small number of ovarioles (six) in the ovary limits the maximum number of eggs per pod, which at least partially constrains overall fecundity. Even so, *A. deorum* dominates at many western US grassland sites, suggesting that mechanisms exist to overcome low reproductive potential (e.g., sustaining high survival or increasing the number of egg pods laid each year).

We collected adult *A. deorum* from field sites near Arapaho Prairie (Arthur County), Neb., USA, during mid and late July 1992, shortly after the adults emerged, and transferred them to the laboratory. Individuals were fed a combination of dry wheat bran and 7 to 10-day-old barley seedlings for the remainder of their life. After collecting eggs, we accommodated the diapause stage by storing eggs at 6°C for 4.5 months. Following diapause, we hatched eggs in an insectary at 30°C with a 14:10 light:dark (L:D) photophase. We placed individual cages housing grasshoppers next to 40-W incandescent light bulbs during the entire photophase. Light bulbs provided a heat source that allowed grasshoppers to self-regulate body temperatures above the ambient temperature of the environmental chamber. Newly hatched nymphs were fed an unlimited, mixed diet of barley seedlings and dry wheat bran throughout the nymphal development period. Males and females were separated at the fifth instar. By feeding nymphal grasshoppers ad libitum on natural foods, we assumed that all grasshoppers acquired the necessary macro- and micronutrients needed for normal development and that they were at a nutritionally similar state at the beginning of experiments. These first-generation eggs approximated the genetic background of field populations. Our treatment of field-collected females to provide eggs for the experiments coupled with random assignment to treatments minimized possible maternal effects.

Experimental design

We examined the performance of *A. deorum* on a range of diets using a randomized complete-block design. Within this design, we manipulated 15 unique treatment combinations of dietary carbohydrates and N as a 3 × 5 factorial design, each treatment combination replicated six times. The three carbohydrate levels (4.3, 16.0, and 26.7% dry weight) and five total N levels (1.0, 2.5, 4.0, 5.5, and 7.0% dry weight) spanned ranges reported in naturally occurring grasses on which *A. deorum* typically feeds (Lyttleton 1973; S. Mole and A. Joern, unpublished data).

Defined diets

Agar-based defined diets (Table 1) containing commercially prepared Purina Horse Charge, casein, sucrose, starch, and cellulose allowed us to quantitatively manipulate food quality. Horse Charge is a horse feed supplement containing high-quality plant protein fortified with vitamins and minerals. Ingredients in Horse Charge include plant protein products, grain products, forage products, processed grain by-products, molasses products, animal fat preserved with ethoxyquin, vitamins A, E, B12 and D3 supplements, riboflavin supplement, calcium pantothenate, calcium carbonate, sodium selenate, dicalcium phosphate, calcium iodate, salt, cobalt carbonate, ferrous carbonate, copper sulfate, manganese oxide, and zinc oxide. Artificial, agar-based diets containing

Table 1 Agar-based artificial diet components and concentrations. Ingredients for a single batch as typically prepared, expressed both as mass (g) and percent dry weight of total, are given to explicitly identify constituents. Protein and carbohydrates were added to the base diet as indicated below, depending on the treatment combination. Cellulose was then added to each diet to bring the total dry mass of each diet to 200 g. Diets were prepared using standard procedures. Agar was dissolved in boiling water and the solution allowed to cool before adding the remaining components to prevent any denaturation

Base ingredients	Mass (g)	Dry weight (%)				
Horse Charge	16.6	33.2				
Ascorbic acid	1.6	0.8				
Methyl-paraben	1.4	0.7				
Agar (corn based)	21.4	10.7				
		Nitrogen				
		1.0%	2.5%	4.0%	5.5%	7.0%
Casein (g)	0	18.75	37.5	56.2	74.5	
Dry weight (%)	0	9.38	18.75	28.13	37.25	
		Carbohydrate				
		4.3%	16.0%	26.7%		
Sucrose (g)	2	8	14			
Dry weight (%)	1	4	7			
Starch (g)	4.67	23.34	37.34			
Dry weight (%)	2.34	11.67	18.67			

only Horse Charge successfully support both development and reproduction in grasshoppers (Yang and Joern 1994a, b).

The 15 unique treatment combinations all contained equal amounts of Horse Charge and sorbic acid so that the concentration of micronutrients was constant across all diets. We also held constant both agar and methyl-paraben (an anti-fungal agent) across all diets. Only levels of carbohydrates, dietary N, and cellulose varied across the 15 diet combinations.

Horse Charge contains 6% (dry weight) of both total N and TNCs (Yang and Joern 1994a). However, the basal amount of Horse Charge in each of the present diets contributed 2% and 1% (dry weight) of the total carbohydrate and N level, respectively. We added casein to elevate N levels and a combination of sucrose and starch to elevate carbohydrate levels (Table 1). Cellulose was added so that dry weight portions (20%) and water content (80%) of all diets were held constant.

Experimental protocol and statistical analyses

As female grasshoppers molted to adults, we individually caged and randomly assigned each individual to 1 of 15 treatment combinations. Cages (8 cm diameter, 11 cm height) were constructed of fine-meshed wire screen and contained an inverted 2-dram vial with drinking water. We fed grasshoppers twice daily for the remainder of their adult lives. Individual cages were placed over cups of sand to facilitate oviposition. All experiments were conducted in an environmental chamber set at 30°C with a photophase of L:D 14:10. Individual cages were placed next to 40-W incandescent light bulbs so that individual grasshoppers could self-regulate body temperatures ('thermophase' L:D 6:18). These light sources were on at the same time as other nonheat source lights.

We paired females with males at the beginning of each trial. However, because we focused on the effects of diet quality on female reproductive performance, we regularly rotated males among treatments to minimize any potential effects of diet on sperm availability. We collected individual eggs at 3-day intervals and incubated them in small containers of vermiculite moistened with 0.5% methyl-paraben at ambient room temperature (23–25°C) for 28 days. By collecting eggs every 3 days, we could assess the number of egg pods produced and estimate the number of days between successive egg pods. After the incubation period, we dissected egg pods and recorded the number of eggs produced. We ended all experiments after 50 days even if the female was still alive.

To evaluate reproductive performance, survivorship, and weight gain, we used ANCOVA (Systat 5.2, Multivariate General Linear Hypothesis, Wilkinson 1992), with initial grasshopper weight as a covariate. Linear and quadratic contrasts allowed us to identify trends in the above responses to dietary N and carbohydrate levels. Tukey's post hoc paired comparisons are shown on the figures where treatment means with different letters are significantly different at the $\alpha = 0.05$ level.

Results

Survivorship and growth

Diet quality exerted a significant effect on survivorship (measured as number of days alive) of newly molted adult females as indicated by a significant N-carbohydrate interaction (Table 2). As shown in Fig. 1a, the highest observed average adult survivorship (50.2 days; 1% N, 26.7% carbohydrate) was 76% greater than the

Table 2 Results of analysis of covariance (with initial grasshopper weight as the covariate) on survival and weight gain of *Ageneotettix deorum* in response to five nitrogen (1.0, 2.5, 4.0, 5.5, and 7.0% dry weight) and three carbohydrate (4.3, 16.0, and 26.7% dry weight) levels. The nitrogen \times carbohydrate interaction was not calculated for weight gain because of an insufficient number of observations

Source	df	Days alive		Weight gain	
		F-ratio	P	F-ratio	P
Nitrogen	4	1.08	NS	5.38	0.002
Linear	1	1.50	NS	0.27	NS
Quadratic	1	1.20	NS	14.48	0.001
Carbohydrate	2	1.56	NS	2.67	NS
Linear	1	3.12	0.04	4.49	0.04
Nitrogen \times carbohydrate	8	2.26	0.03	–	–
Weight	1	0.75	NS	0.02	NS

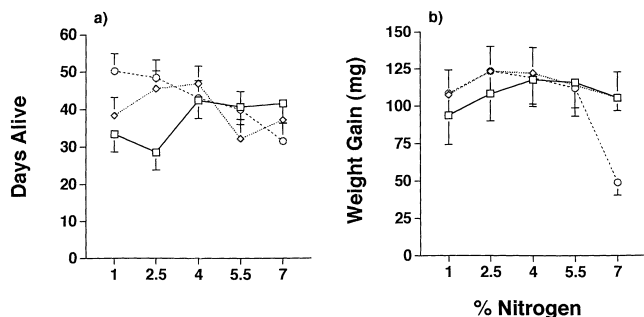


Fig. 1 Average adult survival (a) and weight gain (b) in response to variable total nitrogen and carbohydrates. The three carbohydrate levels (dry weight) are 4.3% (□), 16% (◇), and 26.7% (○)

worst (28.5 days; 2.5% N, 4.3% carbohydrate). Neither N nor carbohydrate was significant as a main effect on adult survivorship although we detected a significant linear contrast for carbohydrates. Survival was lowest when carbohydrate levels and N levels were low and highest when carbohydrate levels were high and N levels low; at higher N levels for all carbohydrate levels, survivorship approached the average (Fig. 1a). The covariate, initial female weight, was not significant (Table 2), indicating that initial size was not strongly correlated with survival independent of diet quality.

A highly significant effect of N existed for weight gain by adult females (Table 2). Moreover, we detected a significant quadratic response as N levels increased (Fig. 1b), with the greatest weight gain occurring at intermediate N levels (2.5 and 4.0%); the average weight gain at 2.5% N (147 mg) was 94.7% greater than the weight gain at 1% N (75.5 mg). The main effect of carbohydrate on weight gain was not significant ($P = 0.08$), but we observed a significant linear increase in weight gain as carbohydrate levels in the diet increased (Table 2), further suggesting their importance. The covariate (initial weight) was not significant.

Reproduction

We analyzed the effect of diet quality on individual egg mass using only egg pods that contained healthy, robust eggs. A significant N-carbohydrate interaction (ANC-

OVA, Table 3) indicated the importance of diet quality to egg mass; we detected no N or carbohydrate main effects. The greatest average egg mass (6.26 mg; 4% N, 4.3% carbohydrate) was 88.6% greater than the smallest egg mass (3.32 mg; 7% N, 4.3% carbohydrate) (Fig. 2a). The interaction can largely be explained by an inverse response in high versus low carbohydrate levels as N concentration increased, the most dramatic differences occurring at 4% and 7% total N (Fig. 2a). Egg mass did not vary much at intermediate levels of carbohydrate for all N levels. Initial grasshopper size did not affect mean egg mass.

N exhibited a highly significant main effect on egg production rate (eggs/day; Table 3) with a highly significant quadratic trend. The greatest average egg

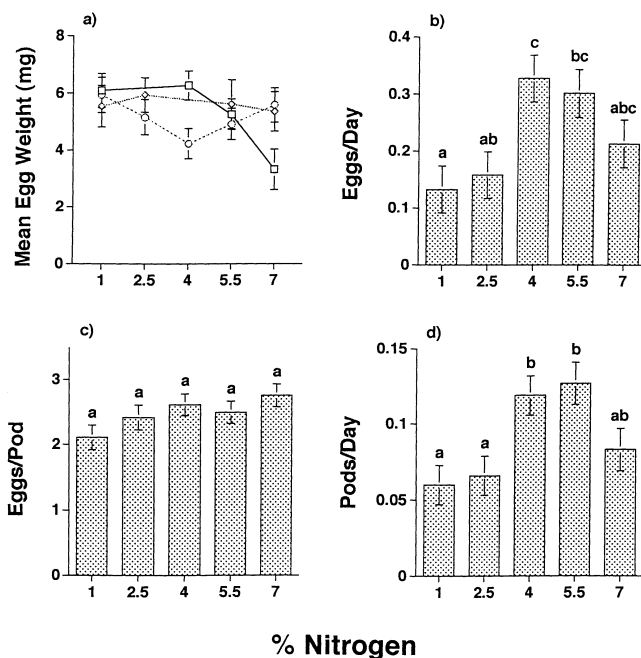


Fig. 2a-d Reproduction in response to total nitrogen levels in the diet. For egg weight, a significant nitrogen × carbohydrate interaction was detected and the three carbohydrate levels are indicated: 4.3% (□), 16% (◇), and 26.7% (○). Dependent variables include mean egg weight (mg) (a), average number of eggs produced per day (b), average number of eggs per pod (c), and average number of pods per day (d), treatment means with different letters are significantly different

Table 3 Results of analysis of covariance (with initial grasshopper weight as covariate) on reproductive responses of *A. deorum* in response to five nitrogen (1.0, 2.5, 4.0, 5.5, and 7.0% dry weight) and three carbohydrate (4.3, 16.0, and 26.7% dry weight) levels

Source	df	Egg weight		Eggs/day		Eggs/pod		Pods/day	
		F-ratio	P	F-ratio	P	F-ratio	P	F-ratio	P
Nitrogen	4	1.44	NS	4.22	0.004	1.79	NS	5.10	0.001
Linear	1	1.80	NS	5.55	0.02	5.78	0.02	6.49	0.01
Quadratic	1	0.29	NS	7.08	0.01	0.35	NS	8.3	0.005
Carbohydrate	2	1.09	NS	1.15	NS	1.24	NS	1.52	NS
Linear	1	0.86	NS	0.43	NS	1.34	NS	2.12	NS
Nitrogen × carbohydrate	8	2.34	0.04	0.62	NS	1.11	NS	0.95	NS
Initial weight	1	2.12	NS	1.25	NS	7.64	0.008	0.05	NS

Table 4 Results of analysis of covariance (with initial grasshopper weight as the covariate) on survival and weight gain of *A. deorum* in response to five nitrogen (1.0, 2.5, 4.0, 5.5, and 7.0% dry weight) and three carbohydrate (4.3, 16.0, and 26.7% dry weight) levels

Source	df	Days to first pod		Days between first and second pod	
		F-ratio	P	F-ratio	P
Nitrogen	4	5.61	0.001	3.76	0.01
Linear	1	8.97	0.004	4.55	0.038
Quadratic	1	11.91	0.001	6.31	0.016
Carbohydrate	2	2.87	NS	2.86	NS
Nitrogen × carbohydrate	8	1.15	NS	2.40	0.03
Weight	1	0.47	NS	1.19	NS

production occurred at intermediate N levels (Fig. 2b). Average egg productivity at 4% N was 1.46 times greater than that observed at the lowest level (1% N). Carbohydrate levels, initial female weight (covariate), and the carbohydrate-N interaction exhibited no significant effect on egg production (Table 3).

N significantly affected the rate of pod production (pods/day) as a main effect (Table 3). The response was quadratic in nature (Fig. 2d), with pod production being greatest at intermediate N levels. The highest average rate of pod production (5.5% N) was 1.2 times faster than production at the lowest rate (1% N). We detected no significant effects of carbohydrate level, initial body size, or the N-carbohydrate interaction on pod production rate.

We also examined clutch size, measured as the number of eggs/pod. It was largely independent of diet quality (Table 3), although there was a significant linear increase in the number of eggs/pod with increased N levels (Fig. 2d). In addition, the number of eggs per pod was positively related to initial female weight, as shown by the significant effect of the covariate (Table 3).

Finally, we examined two aspects of the effects of diet quality on the timing of egg pod production: (a) the elapsed time until the first pod was laid after becoming an adult, and (b) the elapsed time between the first and second egg pods. We observed a significant quadratic (as well as linear) response for N on the number of days until the first pod was laid (Table 4). Elapsed time until the first egg pod was longest on 1% N diets (18.6 days), an increase of about 52% over the average number of days until the first pod for the other dietary N treatments (12.3 days, Fig. 3a). We did not observe a significant effect of carbohydrate, initial body size, or the N-carbohydrate interaction on the number of days until the first egg pod.

Diet quality also affected the average number of days between the first and second egg pod. A significant N-carbohydrate interaction was observed (Table 4). The average interval between the first and second pod was relatively constant on high-carbohydrate diets (mean 4.3 days), whatever the N level, but increased for both low- and medium-carbohydrate diets at low N levels (Fig. 3b). On 1% N diets, the longest interval between pods (15.9 days; 4.3% carbohydrate) was about 1.5 times longer than that for the shortest interval at this N level (6.3 days; 26.6% carbohydrate) and 2.7 times

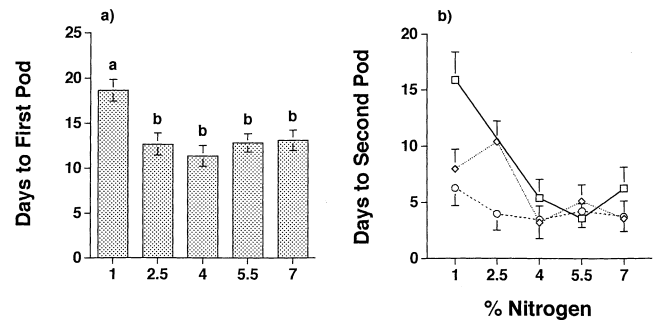


Fig. 3a,b Egg pod production rate in response to diet quality. For days to second pod, a significant nitrogen × carbohydrate interaction was detected and the three carbohydrate levels are indicated: 4.3% (□), 16% (◇), and 26.7% (○). Dependent variables are the number of days from eclosion to the adult stage until the first egg pod (a) and the number of days between the first and second egg pods, if a second egg pod was produced (b). Treatment means with different letters are significantly different.

longer than the average (4.3 days) for high-carbohydrate diets (27.7% carbohydrate, all N levels). Carbohydrate content had little impact on the interval between egg pods at higher N levels (>4%).

Discussion

Importance of dietary N

Many investigators have previously suggested that host plant quality greatly affects survivorship, growth, and reproductive output in many insect herbivores, including grasshoppers (White 1976, 1984; McNeill and Southwood 1978; Rhoades 1979, 1983; Mattson and Haack 1987; Joern and Gaines 1990; Jones and Coleman 1992). Without doubt, diet quality can exert an important influence on egg production, survival, and growth in adult *A. deorum* females. More significant, dietary N appears to be a primary limiting component in the diet of this grasshopper species, suggesting that individuals typically obtain sufficient energy from carbohydrates as they satisfy N needs. Since we chose N-carbohydrate diet combinations in our experiments to reflect a typical range of values observed in naturally occurring host plants, we conclude that dietary N will often be limiting to free-ranging *A. deorum*. Such results

accord well with known responses by many insect herbivores (White 1993).

Compared with carbohydrate, protein contributed most to demographic responses in *A. deorum* females in the present study. Carbohydrate exhibited a significant effect only for survival, adult weight gain, and days between pods, as an interaction with dietary N in each case. Survival and weight gain suggested significant linear trends in response to carbohydrate in the diet. On the other hand, dietary N was significantly implicated in most of the responses (weight gain, eggs/day, eggs/pod, pods/day, days elapsed to first pod, and the interval between pods 1 and 2), and contributed to significant interactions for survival and egg weight. The effect of protein (quantified as N) on these demographic features was typically quadratic, with an optimum response at or near 4–5% total N.

Routine host plant and tissue selection is often based on N content (Haglund 1980; Lewis 1984; Behmer and Joern 1993, 1994). Behavioral and physiological studies of nutrition have repeatedly shown that insect herbivores, including grasshoppers, can readily discriminate among food sources of different quality (Simpson and Abisgold 1985; Simpson and Simpson 1990; Behmer and Joern 1993, 1995). In addition, grasshoppers and some other insects can compensate for imbalances in their diets by choosing food that provides missing nutrients (Simpson and Simpson 1990; Simpson et al. 1990; Waldbauer and Friedman 1991). For example, *Locusta migratoria* nymphs can distinguish the relative amounts of protein and carbohydrate in food (Abisgold and Simpson 1981). When proportions of protein and carbohydrate are varied, nymphs regulate intake based on protein rather than digestible carbohydrates, responses consistent with those of many insect herbivores (White 1993).

While less intensely studied, demographic attributes of adult grasshoppers such as growth rate, developmental rate, survival, and fecundity also routinely vary in response to N levels in the diet, largely in response to internal nutrient economics (Hill et al. 1968; Tobe and Loughton 1969; Lee and Wong 1979; Lim and Lee 1981; Van Heusdon et al. 1987). Growth, and especially survival, in grasshopper nymphs is often protein dependent (McGinnis and Kasting 1961; Lewis 1984; A. Joern and S. Mole, unpublished data). Egg production in several grasshopper species can be quite responsive to changes in dietary N as well (Smith and Northcott 1951; McCaffery 1975). Likewise, switching females from high- to low-protein diets resulted in an immediate decrease in egg production and resorption of terminal oocytes (McCaffery 1975); opposite results were obtained after switching females from low- to high-protein diets.

Reproductive and demographic consequences

Naturally occurring *A. deorum* females in Nebraska sandhills grassland become adults in mid July and begin

laying eggs approximately 1–2 weeks later. Female grasshoppers face a rapidly changing, deteriorating nutritional environment just as they become reproductively mature. This nutritional crunch exerts a significant impact on the key demographic attributes studied here, affecting both individual fitness and population responses. At this point, females have a window of approximately 8–12 weeks in which to lay eggs, in the unlikely event that they live that long. In particular, foliar N levels in host plants generally reach the lowest levels for the season just as the *A. deorum* females are producing their first and second clutch of eggs (A. Joern and S. Mole, unpublished data). In practice, most eggs are produced by early September and few individuals will lay more than two clutches in the field (Joern and Gaines 1990; A. Joern, unpublished data). Most individuals die by late August and suboptimal temperatures for egg production predominate in September and October, severely limiting egg production by the small, remaining adult population (Gaines 1989; A. Joern and L. Kang, unpublished data). In other words, there exists significant selective pressure on *A. deorum* to produce eggs as soon as possible after maturing, a process that is greatly influenced by seasonal trends in host plant quality.

Both allocation of resources to eggs within an egg pod as well as the rate of egg pod production contribute to reproductive potential. A trade-off between the size and number of eggs in an egg pod and body size was evident and was affected by nutritional quality of the food. In many insects, the fitness of individual offspring may be related to egg size (Capinera 1979), and this also holds for grasshoppers (Atkinson and Begon 1987, 1988; Wall and Begon 1987). This becomes significant to population processes if they are largely driven by a small proportion of highly fit individuals rather than merely reflecting the average of all individuals (Lomnicki 1988; Atkinson and Begon 1988). We have no additional information on this point for *A. deorum*.

The interval between pods may prove to be the most important factor influencing individual reproductive potential and resulting population processes. Diet quality, especially total N content, significantly affected the rate of egg pod production. While N levels best predict the rate of egg production, a strong interaction with carbohydrate levels exists. N effects are most pronounced at low carbohydrate levels. Since reproductively mature *A. deorum* females typically encounter rapidly deteriorating food quality, including significant variation in both N and carbohydrate levels, individual egg pod production, and thus total egg production, is likely to exhibit significant between-season and among-year variation.

Methodological points

Three methodological issues invite comment. In this study, we presented excess food to *A. deorum*. Does this

compromise our interpretations for understanding naturally occurring individuals? In our view, ad libitum feeding reasonably mimics natural conditions and represents an appropriate experimental protocol to evaluate our questions. Free-living grasshoppers typically find sufficient food bulk of some type to eat, except possibly at exceptionally high population densities when no food remains. Because of constraints derived from gut volume and food passage rate (Yang and Joern 1994a, b), food nutritional quality rather than total biomass typically becomes the limiting feature under natural conditions. We feel that ad libitum feeding schedules are appropriate.

Second, we did not hold grasshoppers at constant temperatures, but permitted them to self-regulate body temperatures for a large fraction of the day. Most grasshoppers, including *A. deorum*, maintain body temperature near 36–38°C when possible (Chappell and Whitman 1990). The presence of a light bulb next to the cage for 6 h per day allowed grasshoppers to elevate body temperatures to preferred levels during this period, much as they do under field conditions. As such, dynamic metabolic as well as digestive rates can be controlled by the grasshopper, as appropriate (Yang and Joern 1994a, b). While different individuals may not maintain identical body temperatures (resulting in different metabolic rates and energy needs in our experiments), individuals possess more appropriate internal information regarding nutritional needs and likely use this information to regulate homeostatic activities accordingly. We feel that this procedure strengthens our ability to interpret results in terms of nutrient limitation for free-living individuals. The control that we lose by permitting somewhat variable body temperatures (and thus variable metabolic rates) among individuals is offset by the more precise homeostatic control exerted by individuals. This seems important to us when assessing the multi factor, dynamic features surrounding nutrient limitation to explain naturally occurring situations.

Third, we manipulated carbohydrate and dietary N levels in full factorial fashion to compare responses by *A. deorum* to the full range of combinations of these two dietary components. Can grasshoppers expect to encounter food plants in which carbohydrate and protein are uncorrelated or are they more likely to covary in some systematic fashion? If carbohydrates and dietary N naturally covary in some predictable fashion, grasshoppers may not have evolved mechanisms appropriate for processing completely unique combinations that we may have presented (especially of the high/low variety), resulting in misleading responses by grasshoppers with inappropriate interpretations on our part. At least for common grasses at Arapaho Prairie in Nebraska sandhills grassland, we find no predictable correlation between these two dietary components based on a long-term study (12 years) of seven dominant graminoids (A. Joern and S. Mole, unpublished data). Depending on the year or season, a grasshopper may encounter host plants with high or low levels of both nutritional con-

stituents or some mixed combination. Thus, a factorial design for presenting the nutritional components is the most appropriate way to assess demographic responses to the potential nutritional landscape encountered by grasshoppers.

Significance to demographic processes

Our results support the notion that protein rather than carbohydrate concentrations in host plants limit *A. deorum*, with significant consequences for setting demographic parameters. Key features of growth, survival, and egg production vary in response to N content in the diet. In turn, these determine both the number and quality of individuals in the present generation as well as the potential number in the next. While carbohydrates are clearly required to supply energy, it appears that sufficient energy is obtained if enough N is ingested to satisfy needs; demographic responses suggest that an optimal level occurs at about 4% total N. Our results regarding N limitation for *A. deorum* conform to conclusions of others based on both ecological (White 1993) and physiological (Hinks et al. 1993) assumptions. This is important, because N levels in many food plants are highly variable in natural systems and respond quickly to many environmental stresses (White 1984; Mattson and Haack 1987; Jones and Coleman 1992).

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References

- Abisgold JD, Simpson SJ (1987) The physiology of compensation by locusts for changes in dietary protein. *J Exp Biol* 129:329–346
- Abisgold JD, Simpson SJ, Douglas AE (1994) Nutrient regulation in the pea aphid *Acyrtosiphon pisum*: application of a novel geometric framework to sugar and amino acid consumption. *Physiol Entomol* 19:95–102
- Atkinson D, Begon M (1987) Ecological correlates and heritability of reproductive variation in two co-occurring grasshopper species. *Ecol Entomol* 12:129–138
- Atkinson D, Begon M (1988) Adult size variation on two co-occurring grasshopper species in a sand-dune habitat. *J Anim Ecol* 57:185–200
- Behmer ST, Joern A (1993) Dietary selection by the generalist grasshopper *Phoetaliotes nebrascensis* (Orthoptera: Acrididae). *Funct Ecol* 7:522–527
- Behmer ST, Joern A (1994) The influence of proline on diet selection: sex-specific feeding preferences by the grasshoppers *Ageneotettix deorum* and *Phoetaliotes nebrascensis* (Orthoptera: Acrididae) based on the need for phenylalanine. *Oecologia* 98:76–82
- Bernays EA (1982) The insect on the plant – a closer look. In: Visser JH, Minks AK (eds) *Proceedings 5th international*

- symposium on insect-plant relationships. Pudoc, Wageningen, pp 3–17
- Bloom AJ, Chapin FS III, Mooney HA (1985) Resource limitation in plants – an economic analogy. *Annu Rev Ecol Syst* 16:363–392
- Capinera J (1979) Qualitative variation in plants and insects: effect of propagule size on ecological plasticity. *Am Nat* 114:350–361
- Cappuccino N, Price PW (eds) (1995) Population dynamics. Academic Press, San Diego
- Chappell MA, Whitman DW (1990) Grasshopper thermoregulation. In: Chapman RF, Joern A (eds) *Biology of grasshoppers*. Wiley, New York, pp 143–172
- Dadd RH (1960) Observations on the palatability and utilization of food by locusts, with particular reference to the interpretation of performance in growth trials using synthetic diets. *Entomol Exp Appl* 3:283–304
- Dadd RH (1985) Nutrition: organisms. In: Kerkut GA, Gilbert LI (eds) *Comparative insect physiology, biochemistry and pharmacology*, vol 4. Pergamon, Oxford, pp 177–217
- Gaines SB (1989) Experimental analysis of costs and benefits of wing length polymorphism in grasshoppers. PhD thesis, University of Nebraska-Lincoln
- Haglund BM (1980) Proline and valine – cues which stimulate grasshopper herbivory during drought stress? *Nature* 288:697–698
- Hill CF, Luntz AJ, Steele PA (1968) The relationships between somatic growth, ovarian growth and feeding activity in the adult desert locust. *J Insect Physiol* 14:1–20
- Hinks CF, Cheeseman MT, Erlandson MA, Olfert O, Westcott ND (1991) The effects of kochia, wheat and oats on the digestive proteinases and the protein economy of adult grasshopper, *Melanoplus sanguinipes*. *J Insect Physiol* 37:417–430
- Hinks CF, Hupka D, Olfert O (1993) Nutrition and the protein economy in grasshoppers and locusts. *Comp Physiol* 104A:133–142
- Joern A (1979) Feeding patterns in grasshoppers (Orthoptera: Acrididae): factors influencing diet specialization. *Oecologia* 38:325–347
- Joern A (1985) Grasshopper dietary from a Nebraska sand hills prairie. *Trans Neb Acad Sci* 8:21–32
- Joern A, Gaines SB (1990) Population dynamics and regulation in grasshoppers. In: Chapman RF, Joern A (eds) *Biology of grasshoppers*. Wiley, New York, pp 415–482
- Jones CG, Coleman JS (1992) Plant stress and insect herbivory: toward an integrated perspective. In: Mooney HA, Winner WE, Pell EJ (eds) *Response of plants to multiple stresses*. Academic Press, San Diego, pp 249–280
- Lee SS, Wong IM (1979) The relationship between food plants, haemolymph protein and ovarian development in *Oxya japonica* (Acrididae: Orthoptera). *Acrida* 8:1–8
- Lewis AC (1984) Plant quality and grasshopper feeding: effects of sunflower condition on preference and performance in *Melanoplus differentialis*. *Ecology* 65:836–843
- Lim SJ, Lee SS (1981) The effect of starvation on haemolymph metabolites, fat body and ovarian development in *Oxya japonica* (Acrididae: Orthoptera). *J Insect Physiol* 27:93–96
- Lomnicki A (1988) *The population ecology of individuals*. Princeton University Press, Princeton, NJ
- Lyttleton JW (1973) Amino acids, peptides and ureides. In: Butler G, Bailey L (eds) *Chemistry and biochemistry of herbage*. Academic Press, New York, pp 63–103
- Mattson WJ Jr (1980) Herbivory in relation to plant nitrogen content. *Annu Rev Ecol Syst* 11:119–161
- Mattson WJ, Haack RA (1987) The role of drought in outbreaks of plant-eating insects. *Bioscience* 37:110–118
- McCaffery AR (1975) Food quality and quantity in relation to egg production in *Locusta migratoria migratorioides*. *J Insect Physiol* 21:1551–1558
- McGinnis AJ, Kasting R (1967) Dietary cellulose: effect on food consumption and growth of a grasshopper. *Can J Zool* 45:365–367
- McNeill S, Southwood TRE (1978) The role of nitrogen in the development of insect/plant relationships. In: Harborne J (ed) *Biochemical aspects of plant and animal coevolution*. Academic Press, London, pp 77–98
- Mole S, Zera A (1993) Differential allocation of resources underlies the dispersal-reproduction trade-off in the wing polymorphic cricket, *Gryllus rubens*. *Oecologia* 93:121–127
- Rhoades DF (1979) Evolution of plant chemical defenses against herbivores. In: Rosenthal GA, Jansen DH (eds) *Herbivores*. Academic Press, New York, pp 3–54
- Rhoades DF (1983) Herbivore population dynamics and plant chemistry. In: Denno RF, McClure MS (eds) *Variable plants and herbivores in natural and managed systems*. Academic Press, New York, pp 155–220
- Roff DA (1992) *The evolution of life histories*. Chapman and Hall, New York
- Scriber M (1984) Host-plant suitability. In: Bell WJ, Carde RT (eds) *Chemical ecology of insects*. Sinauer, Sunderland, Mass, pp 159–202
- Simpson SJ, Abisgold JD (1985) Compensation by locusts for changes in dietary nutrients: behavioral mechanisms. *Physiol Entomol* 10:443–452
- Simpson SJ, Simpson CL (1990) The mechanisms of nutritional compensation by phytophagous insects. In: Bernays EA (ed) *Insect-plant interactions*, vol 2. CRC, Boca Raton, Fla, pp 111–160
- Simpson SJ, Simmonds SS, Blaney WM, Hones JP (1990) Compensatory dietary selection occurs in larval *Locusta migratoria* but not *Spodoptera littoralis* after a single deficient meal during ad libitum feeding. *Physiol Entomol* 15:235–242
- Simpson SJ, Abisgold JD, Douglas AE (1995) Response of the pea aphid (*Acyrtosiphon pisum*) to variation in dietary levels of sugar and amino acids: the significance of amino acid quality. *J Insect Physiol* 41:71–75
- Slansky F (1982) Insect nutrition: an adaptationist's perspective. *Florida Entomologist* 65:45–71
- Smith DS, Northcott FE (1951) The effects on the grasshopper *Melanoplus mexicanus mexicanus* (Sauss.) (Orthoptera: Acrididae) of varying the nitrogen content of its food plant. *Can J Zool* 29:297–304
- Tobe SS, Loughton BG (1969) An investigation of haemolymph protein economy during fifth instar of *Locusta migratoria migratorioides*. *J Insect Physiol* 15:1659–1672
- Van Heusden MC, Van der Horst DJ, Voshol J, Beenackers AM (1987) The recycling of protein components of the flight-specific lipophorin in *Locusta migratoria*. *Insect Biochem* 17:771–776
- Waldbauer GP, Friedman S (1991) Self-selection of optimal diets by insects. *Annu Rev Entomol* 36:43–63
- Wall R, Begon M (1987) Population density, phenotype and reproductive output in the grasshopper *Chorthippus brunneus*. *Ecol Entomol* 12:331–339
- White TCR (1976) Weather, food and plagues of locusts. *Oecologia* 22:119–134
- White TCR (1984) The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63:90–105
- White TCR (1993) *The inadequate environment: nitrogen and the abundance of animals*. Springer, Berlin Heidelberg New York
- Wilkinson L (1992) *SYSTAT: the system for statistics*. SYSTAT Inc, Evanston, Ill
- Yang Y, Joern A (1994a) Compensatory feeding in response to variable food quality by *Melanoplus differentialis*. *Physiol Entomol* 19:75–82
- Yang Y, Joern A (1994b) Influence of diet, developmental stage and temperature on food residence time. *Physiol Zool* 67:598–616