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Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth

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Summary

We examined correlates of nutrient balancing with dietary range by comparing diet selection and ingestive, post-ingestive and performance-related responses to macronutrient imbalance in two species of grasshopper. One of the two species, *Locusta migratoria* (the African migratory locust), is a specialist grass-feeder, while the other, *Schistocerca gregaria* (the desert locust), is a generalist herbivore that includes both grasses and forbs in its diet. In *ad libitum* conditions, both species composed a balanced intake of the two macronutrients protein and carbohydrate from nutritionally complementary synthetic foods, but the composition of the selected diet differed, with the generalist selecting more protein, but not carbohydrate, than the grass-specialist. The grass-specialist, by contrast, retained ingested nitrogen more efficiently on the *ad libitum* diets. When confined to nutritionally imbalanced foods, both species regulated ingestion in such a way as to mitigate excesses as well as deficits of the two nutrients. The responses were, however, distinct in the two species, with the generalist feeder ingesting greater excesses of protein than the specialist. The species also differed in their post-ingestive responses to ingested excesses of nutrient, with the generalist but not the specialist using protein-derived carbon as an energy source when fed carbohydrate-deficient foods. The generalist also retained a higher level of body protein when confined to protein-deficient diets. The data suggested one functional reason why the generalist species selected a diet with higher protein content in the *ad libitum* treatment because, when confined to the nutritionally imbalanced foods, development rate peaked on higher protein foods for the generalist compared with the specialist. Many aspects of these data agree with the prediction that generalist-feeding animals should show greater behavioural and physiological flexibility in their responses to nutrient imbalance than do specialists.

Key words: nutrient balancing, *Locusta migratoria*, *Schistocerca gregaria*, locust, dietary range, herbivore nutrition, macronutrient.

Introduction

The question of which selective factors have driven the evolution of host selection and host range by insect herbivores is currently an area of active interest. Although overtly ecological factors such as susceptibility to predation and other aspects of habitat association undoubtedly play a role, there is widespread consensus that plant chemistry is central (Slansky and Rodriguez, 1987; Schultz, 1988; Ehrlich and Murphy, 1988; Courtney, 1988; Rausher, 1988; Bernays and Graham, 1988; Bernays and Chapman, 1994; Schoonhoven et al., 1998; Ananthakrishnan, 2001).

The most extensively researched group of chemicals that have been studied in this context are the non-nutrient allelochemicals (Ehrlich and Raven, 1964; Rosenthal and Berenbaum, 1992; Farrell and Mitter, 1998; Berenbaum, 2001; Mauricio, 2001). Surprisingly little information exists, by contrast, on the role of nutrients in host selection and the evolution of host range in herbivorous insects – despite the self-evident truth that in most instances nutrition is the *raison d'être* for the association between a phytophagous insect and its host plants. The expectation that nutrient content might be an important factor in the patterns of host selection by phytophagous insects is reinforced by the knowledge that great variation exists in the nutrient content of plants, both in space and time (Osier and Lindroth, 2001; von Fircks et al., 2001; Lindroth et al., 2002; Gusewell and Koerselman, 2002; Oleksyn et al., 2002), and that insects are susceptible to such variation (Scriber and Slansky, 1981; Slansky and Rodriguez, 1987; Bernays and Chapman, 1994; Raubenheimer and Simpson, 1997; Schoonhoven et al., 1998). It has also been suggested that the patterns of host selection in phytophagous insects might have influenced the macronutrient content of their plants, in a process analogous to coevolution of insects with defensive plant-produced allelochemicals (Moran and Hamilton, 1980; Lundberg and Astrom, 1990; Augner, 1995; Berenbaum, 1995).

One component of nutritional variability that might play a role is the concentration of nutrients in relation to non-utilisable bulk such as cellulose (Abe and Higashi, 1991; Hochuli, 1996). It has been reported that herbivores sometimes avoid plant parts that contain a high proportion of structural compounds (Choong et al., 1992; Williams et al., 1998), but the interpretation of this remains unclear because, in addition to affecting nutrient concentration, structural compounds influence leaf toughness (Sands and Brancatini, 1991; Choong et al., 1992; Hochuli, 1996). Furthermore, experiments that separate out the mechanical from the dilution effects of plant bulk components using artificial diets have demonstrated that herbivorous insects have a well-developed capacity to compensate for nutrient dilution by increasing the amount of food processed (Simpson and Simpson, 1990; Raubenheimer and Simpson, 1993), and the same has been demonstrated using real plant tissue (Slansky and Feeny, 1977; Simpson and Simpson, 1990). Plants might also be qualitatively deficient relative to an insect's nutritional requirements, such that one or more essential nutrients is lacking or present in a non-utilizable form. For example, insects lack the ability to synthesise sterols, and some plants contain sterols only in a form that cannot be utilised by insects (Behmer and Grebenok, 1998; Behmer and Elias, 1999).

The component of plant variation that, until recently, has received very little attention in relation to host range in herbivorous insects is the balance of macronutrients. This is notwithstanding the existence of good reasons for suspecting an important role for macronutrient balance. Comparative analysis has revealed, for example, that insects differ widely in the balance of protein and digestible carbohydrate that gives optimal performance (Simpson and Raubenheimer, 1993), and fitness costs can be pronounced for insects feeding on foods that diverge from the required balance (e.g. Slansky and Feeny, 1977; Raubenheimer and Simpson, 1997; Joern and Behmer, 1997). Unlike nutrient dilution (Simpson and Simpson, 1990), nutritional imbalance cannot easily be compensated for, because any increased consumption of the deficient nutrient(s) in an imbalanced food entails ingesting excesses of others, and existing data suggest that many animals have a limited capacity to ingest nutrient excesses (Raubenheimer, 1992; Raubenheimer and Simpson, 1997, 1999). Unsurprisingly, therefore, feeding on nutritionally imbalanced foods can have fitness costs for insects that are avoided when feeding on balanced but nutritionally dilute foods (Raubenheimer and Simpson, 1993, 1997). An animal can, however, utilise imbalanced foods by incorporating them into a broader diet together with other foods that contain complementary nutrient imbalances (Rapport, 1980; Chambers et al., 1995; Simpson and Raubenheimer, 1995; Raubenheimer and Simpson, 1997).

From the viewpoint of macronutrient balance there are thus grounds to suspect that there may be two nutritional strategies that represent extremes in a continuum in host range selection: specialists, which feed on a narrow range of tissues that closely approximate the required balance of macronutrients, and generalists, which compose a diet from a wider range of nutritionally complementary foods. Alternatively, it might be that insects that are food plant generalists are in fact *nutrient* specialists, in that a wide host range better enables them to defend a balanced diet than plant specialists whose nutrient intake is more vulnerable to variation in a narrow range of foods (Raubenheimer and Simpson, 1999). In either event, the ability to tolerate a sub-optimal balance of ingested nutrients would require appropriate post-ingestive regulatory responses, such as an ability to selectively excrete or store ingested excesses. Unfortunately, these relationships remain obscure, owing to a lack of data relating host range in herbivorous insects to macronutrient intake and post-ingestive regulatory responses.

We have recently initiated a programme to explore these issues by comparing in closely related pairs of generalist- and specialist-feeding insects the diet selection and ingestive, post-ingestive and performance-related responses to macronutrient imbalance. Previously, we have compared the solitarious and gregarious phenotypes of the desert locust, which are genetically identical but, due to their differing ecological circumstances, are likely to encounter a different range of host plants (Simpson et al., 2002). We found that the two morphs have similar optimum macronutrient requirements but that they respond very differently when confined to nutritionally imbalanced foods. Specifically, the gregarious morph, which is highly mobile and has a broader host range than the more sessile solitarious form, ingests greater excesses of the surplus nutrient in imbalanced foods. These data support the hypothesis that plant generalists are opportunistic in acquiring nutrient excesses when available and use them to complement imbalances that might exist in foods that are subsequently encountered (Raubenheimer and Simpson, 1999; Simpson et al., 2002). Here, we performed a detailed comparison of the ingestive, post-ingestive and developmental responses of the gregarious form of two species of grasshopper that differ in their host range: the generalist-feeding *Schistocerca gregaria* and the grass-specialist *Locusta migratoria*.

Materials and methods

Insects

Experimental locusts (*Locusta migratoria* L. and *Schistocerca gregaria* Forskäl) were crowd-reared for many generations (since 1983) in large breeding bins, where they had *ad libitum* access to greenhouse-grown seedling wheat and wheat germ. Nymphs of both sexes were collected from the cultures at ecdysis to the fifth stadium (day 0). Each locust was weighed and placed alone into a 28 cm×15 cm×8 cm clear plastic arena. One or two food dishes were provided, these being modified 5.5-cm Petri dishes filled with approximately 2 g of synthetic food (Raubenheimer and Simpson, 1990). A tissue culture flask perforated with a 1.5-cm hole provided a water source. Insects were kept at 29–31°C under a 12 h:12 h light:dark regime.

Diets

Locusts were allocated to one of six diet treatments. Six of these comprised a single food, varying in the ratio of protein to digestible carbohydrate as follows: 7% protein with 35% digestible carbohydrate (7:35), 14:28, 21:21, 28:14, 35:7 and 42:0. The remaining treatment was given two nutritionally complementary foods (28:14 and 14:28) simultaneously, and so allowed to compose a diet of prefered protein:carbohydrate balance. The dry, granular, synthetic foods were based on those described by Simpson and Abisgold (1985). All foods contained 54% cellulose powder and 4% essential micro-nutrients (salts, vitamins, cholesterol and linoleic acid). Digestible carbohydrate consisted of a 1:1 mix of sucrose and white dextrin, while the protein contained 3:1:1 casein/peptone/albumen.

Protocol

Representatives of all treatments were run concurrently, with the experiment being replicated twice to yield a total of 10 locusts per treatment. Dry mass of food consumed (mass change in the food dishes) was recorded over the first 3 days, 5 days and until adult ecdysis. From this, the amounts of protein and carbohydrate consumed could be calculated. Additionally, the duration of the 5th stadium was recorded to the nearest day. Upon moulting to adults, the insects were frozen and dried to constant mass in a desiccating oven at 40°C. Carcasses were weighed to the nearest 0.1 mg and then lipid-extracted in three 24-h changes of chloroform before being re-dried and re-weighed to give lipid content. The lipid-free carcasses were then analysed for nitrogen content using the micro-Kjeldahl procedure as in Simpson et al. (2002).

Statistical analysis

Unless otherwise stated, data analysis was undertaken using the General Linear Model facility in SPSS (version 9.0). Details of models and transformations are provided in the relevant sections of the Results. Rates and efficiencies were analysed by combining analysis of covariance (ANCOVA) and graphical analysis, to avoid the statistical and interpretive problems associated with ratio-based nutritional indices (Raubenheimer and Simpson, 1992, 1994; Raubenheimer, 1995).

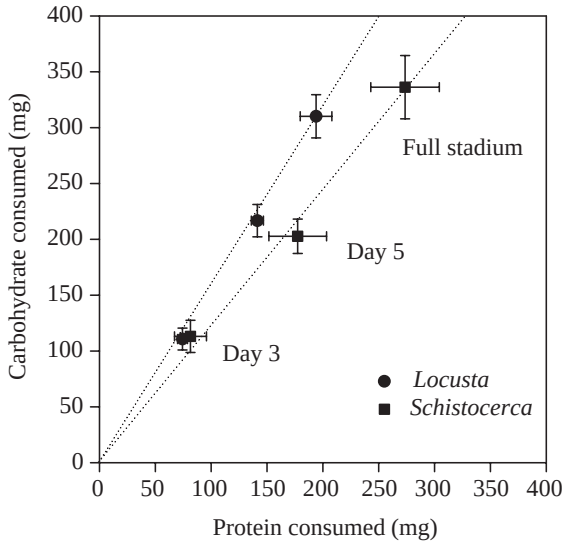

Fig. 1. Bi-variate selected intake point by *L. migratoria* and *S. gregaria* over the first 3 days and 5 days of the final larval stadium and across the entire stadium.

Results

Selected diet

Stadium duration

In the choice treatment, the duration of the 5th stadium was significantly longer in *Schistocerca* (11.5±0.47 days) than in *Locusta* (9.8±0.39 days; $F_{1,13}$=8.39, P=0.012, N=10), but there was no significant species × sex interaction ($F_{1,13}$=0.05, P=0.836, N=10).

Nutrient intake

Fig. 1 shows the protein and carbohydrate intake selected by *Locusta* and *Schistocerca* over the first 3 days and 5 days of the 5th larval stadium and across the entire stadium; statistical analyses are presented in Table 1. Nutrient consumption by the two species was indistinguishable on day 3 but thereafter progressively diverged, with the result that, across the stadium, *Schistocerca* had selected an intake point significantly higher in protein, but not carbohydrate, than had *Locusta*. The greater

Table 1. *F ratios for General Linear Model analysis of protein and carbohydrate (Cho) intake across the first 3 days, 5 days and the entire 5th stadium of male and female* Locusta migratoria *and* Schistocerca gregaria *with a choice of complementary foods*

Dependent variable	Day 3		Day 5		Full stadium			
	Protein†	Cho†	Protein†	Cho	Protein	Cho	Protein	Cho
Covariate								
Stadium duration	–	–	–	–	–	–	0.227	0.395
Factors								
Species	0.195	0.072	1.218	0.281	14.352**	3.214	10.27**	1.014
Sex	0.122	1.086	1.602	1.947	13.158**	30.325***	11.309**	20.59***
Species × sex	0.631	0.617	0.055	0.020	1.509	0.868	1.366	0.367

*, 0.05>P>0.01; **, 0.01>P>0.001; ***, P<0.001; –, terms excluded from the model.
†Rank transformed.

Table 2. *F ratios for General Linear Model analysis of measured carcass mass components, and their relationship with protein and carbohydrate intake (i.e. retention efficiencies) of newly moulted adult* Locusta migratoria *and* Schistocerca gregaria *with a choice of complementary foods*

Dependent variable	Dry carcass mass	Carcass nitrogen		Carcass lipids†		Unaccounted carcass mass†	
Covariates							
Protein intake	–	–	127.89***	–	1.088	–	12.206**
Cho intake	–	–	–	–	0.488	–	2.600
Factors							
Species	14.034**	5.309*	12.530**	0.494	0.923	7.986*	0.114
Sex	23.608***	19.799***	6.283*	4.655*	1.085	12.745**	0.213
Species × sex	4.244	1.891	0.184	0.202	0.325	1.139	0.152

*, 0.05>*P*>0.01; **, 0.01>*P*>0.001; ***, *P*<0.001; –, terms excluded from the model.
†Rank transformed.

protein intake by *Schistocerca* could not be accounted for by differences in stadium duration, as the species term remained significant when stadium duration was entered into the model as a covariate (Table 1). There were no significant species × sex interactions.

Body composition and retention efficiencies

Schistocerca had significantly greater dry body mass than *Locusta* (mean ± S.E.M., 331±14.0 mg *vs* 263±11.6 mg; see Table 2 for statistical comparisons), with no significant species × sex interaction. Similarly, body nitrogen was higher in *Schistocerca* (36.6±1.81 mg) than in *Locusta* (31.2±1.50 mg), again with no significant interaction term.

To test whether the difference in carcass nitrogen was due to differences in consumption or processing efficiencies, protein intake was entered as a covariate into the model (Table 2). Not surprisingly, body nitrogen was strongly related to protein intake. Additionally, the main effect of species remained significant once protein intake had been taken into account, suggesting that the species differed in the efficiency of nitrogen utilisation. Fig. 2A shows that *Locusta* retained ingested nitrogen with greater efficiency than did *Schistocerca* (marginal means ± S.E.M., 34.4±0.468 mg for *Locusta* and 31.2±0.633 mg for *Schistocerca*).

There were no significant differences between the two species in body lipid content, either as a main effect or in interaction with sex (Table 2).

In addition to nitrogen and lipid, we analysed the component of body mass that was not due to nitrogen or lipid, calculated as: total dry body mass – (lipid + nitrogen). This unaccounted mass was significantly greater for *Schistocerca* (243.1±22.41 mg) than for *Locusta* (191.2±9.90 mg), with no significant species × sex interaction (Table 2). ANCOVA revealed that this component of body mass was strongly related to protein intake (Fig. 2B) but not to carbohydrate intake, with no residual sex or species difference (Table 2).

To test for concentration differences in body nitrogen, an ANCOVA was performed using body nitrogen as dependent variable and nitrogen-free carcass mass as covariate (Table 3). Carcass mass correlated strongly with carcass nitrogen content but there was no residual species effect. This suggests that the significant species effect in the analysis of variance (ANOVA) of carcass nitrogen (above) is due to the larger overall mass of *Schistocerca* compared with *Locusta* rather than a higher concentration of nitrogen.

However, a more detailed picture can be obtained by analysing the relationship between nitrogen content and individual components of body mass. There was no significant effect of carcass lipid content as a covariate on carcass nitrogen, and species remained significant in this model (Table 3). There was, however, a strongly significant correlation between unaccounted body mass and carcass nitrogen, but the species term remained significant suggesting that unaccounted body mass alone could not explain differences in carcass nitrogen. Fig. 2C shows that the basis for this effect was a lower nitrogen content per unit of unaccounted body mass in the tissues of *Schistocerca* (marginal means ± S.E.M., 31.1±0.95 mg) than of *Locusta* (34.3±0.69 mg). Therefore, whereas *Schistocerca* had higher levels of nitrogen in the carcass, the concentration of nitrogen

Table 3. *F ratios for General Linear Model analysis of the relationship between nitrogen content and other mass components of newly moulted adult* Locusta migratoria *and* Schistocerca gregaria *with a choice of complementary foods*

Dependent variable	Carcass nitrogen content		
Covariates			
Nitrogen-free carcass mass	13.925**	–	–
Carcass lipids	–	0.625	–
Unaccounted carcass mass	–	–	74.667***
Factors			
Species	0.234	5.596*	5.566*
Sex	0.795	18.8***	0.421
Sex × species	0.036	2.222	0.813

*, 0.05>*P*>0.01; **, 0.01>*P*>0.001; ***, *P*<0.001; –, terms excluded from the model.

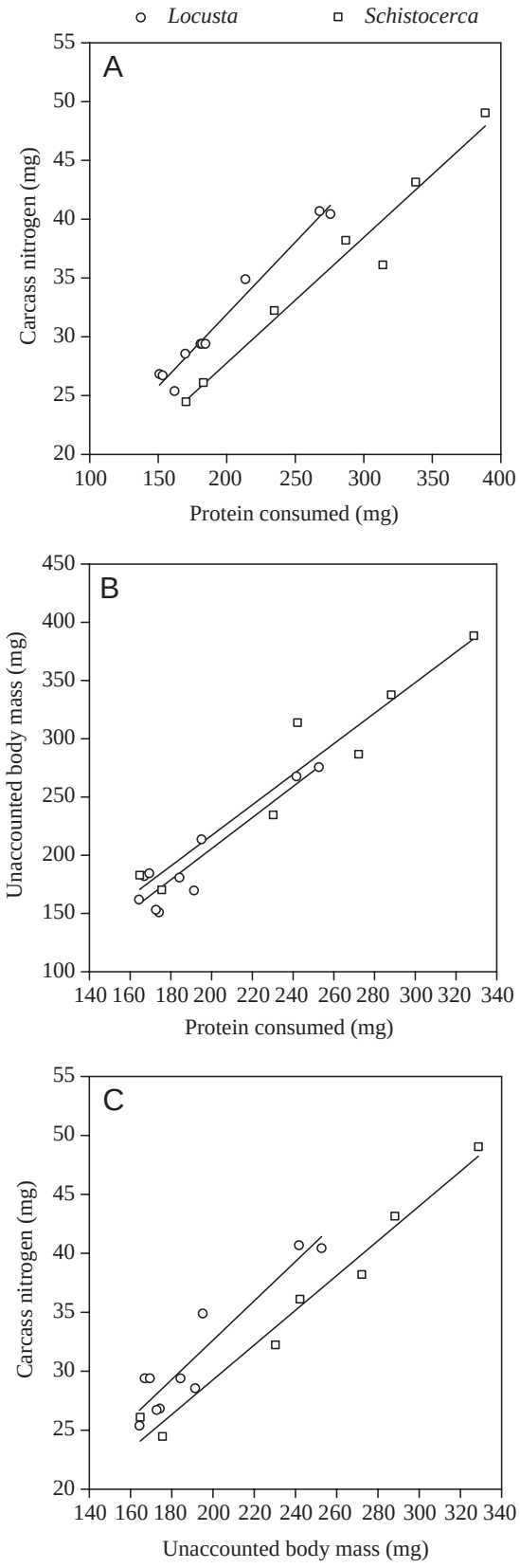

Fig. 2. Relationship between protein consumed and carcass nitrogen (A), protein consumed and unaccounted body mass (carcass dry mass minus nitrogen and lipid) (B), and unaccounted body mass and carcass nitrogen (C) in larval *L. migratoria* and *S. gregaria.*

in the tissues per unit of unaccounted body mass was lower than in *Locusta*.

While we did not directly characterise the unaccounted portion of body mass, its strong dependence on protein intake (above) suggests that it may consist largely of the non-nitrogen component of amino acids. This is borne out by the fact that the observed ratios of unaccounted body mass to N for both species were very similar to the generalised value of 6.25 for non-nitrogen components of protein:nitrogen (Long, 1971). Indeed, for *Locusta*, the value was statistically indistinguishable from the expected value (6.21±0.12; P=0.49, N=10; two-tailed one-sample t-test), while for *Schistocerca* the value was slightly but significantly higher (i.e. the proportion of nitrogen was lower) than expected (6.80±0.12; P=0.003, N=10).

Imbalanced foods

Stadium duration

Stadium duration increased with dietary imbalance in both species, and *Schistocerca* experienced slower development on most diets compared with *Locusta* (Fig. 3). A significant diet × species interaction (Table 4) suggested that the response to dietary imbalance differed between the species. From Fig. 3, it can be seen that the basis for this interaction was, firstly, a shift in the response curve of *Schistocerca* to the right, such that in this species the most rapid development was observed on higher protein diets compared with *Locusta*. Secondly, *Locusta* experienced a disproportionately large increase in stadium duration on diet 42:0. Means in the figure give the impression that, conversely, *Schistocerca* experienced a disproportionate increase in stadium duration on diet 7:35. This was, however, not the case, as the mean stadium duration for *Schistocerca* on this diet was heavily influenced by a single animal with a stadium duration of 33 days, compared with a mean of 21 days

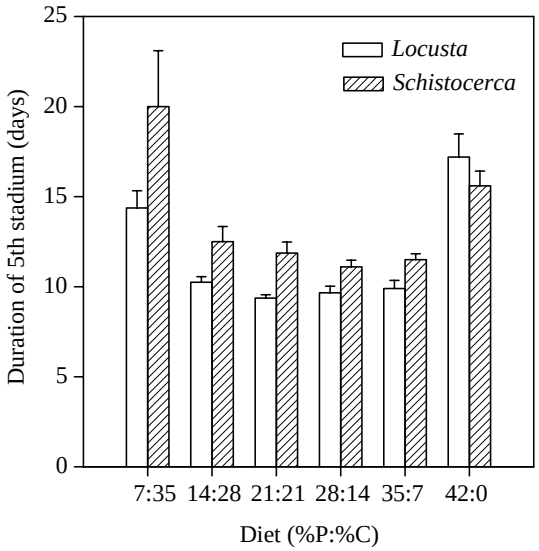

Fig. 3. Duration of the 5th larval stadium in *L. migratoria* and *S. gregaria* fed one of a range of diets differing in protein:carbohydrate (P:C) ratio.

Table 4. *F ratios for analysis of variance (ANOVA) on the duration of the 5th larval stadium in* Locusta migratoria *and* Schistocerca gregaria *fed one of a range of nutritionally imbalanced diets*

Dependent variable	Stadium duration	
	6 diets†	5 diets
Factors		
Diet	31.776***	42.99***
Species	44.013***	19.235***
Sex	8.663**	17.44***
Diet × species	2.891*	4.072**
Diet × sex	1.045	3.566*
Sex × species	1.394	0.008
Diet × sex × species	1.085	0.826

The first analysis includes all six diets, while the second analysis excludes diet 7:35, which included extreme outliers (see Fig. 4).

*, $0.05>P>0.01$; **, $0.01>P>0.001$; ***, $P<0.001$.

†Rank transformed.

for the remainder of animals in this group (the cause of the inflated standard error for this group). Excluding diet 7:35 normalised variances, enabling analysis of untransformed data, and in fact increased the strength of the diet × species interaction term (Table 4). This demonstrates that the main contribution to the analysis of the data for *Schistocerca* on diet 7:35 was the large variance rather than the high mean.

Nutrient intake

Bivariate plots showing nutrient intake across the first 3 days and the first 5 days of the 5th stadium and across the entire stadium are shown in Fig. 4. On days 3 and 5, the pattern for the grass-feeding *Locusta* was curved, resembling the closest distance configuration of intake points previously observed for this species (Raubenheimer and Simpson, 1993). By contrast, the configuration for *Schistocerca* was more linear, resembling the equal distance configuration (Raubenheimer and Simpson, 1997, 1999). Since no transformation could be found that homogenised variances in the intake of protein and carbohydrate, reliable multifactorial tests could not be performed on the data for days 3 and 5. Therefore, separate *t*-tests (assuming unequal variances) were used for each diet, comparing the distance moved along the respective rails by the two species (this distance is calculated using Pythagoras's theorem, as $\sqrt{p^2+c^2}$, where p and c are the amounts of protein and carbohydrate eaten, respectively). These tests showed that, for both days 3 and 5, *Schistocerca* moved significantly further than did *Locusta* along rails with an extreme excess of protein (35:7 and 42:0), and so ingested more of both nutrients, but the difference diminished progressively with increasing proportion of carbohydrate in the diets (Fig. 4A,B).

Across the full stadium, the relationship between diet and the intake of protein and carbohydrate could be linearised by transforming these variables. This enabled us to analyse the

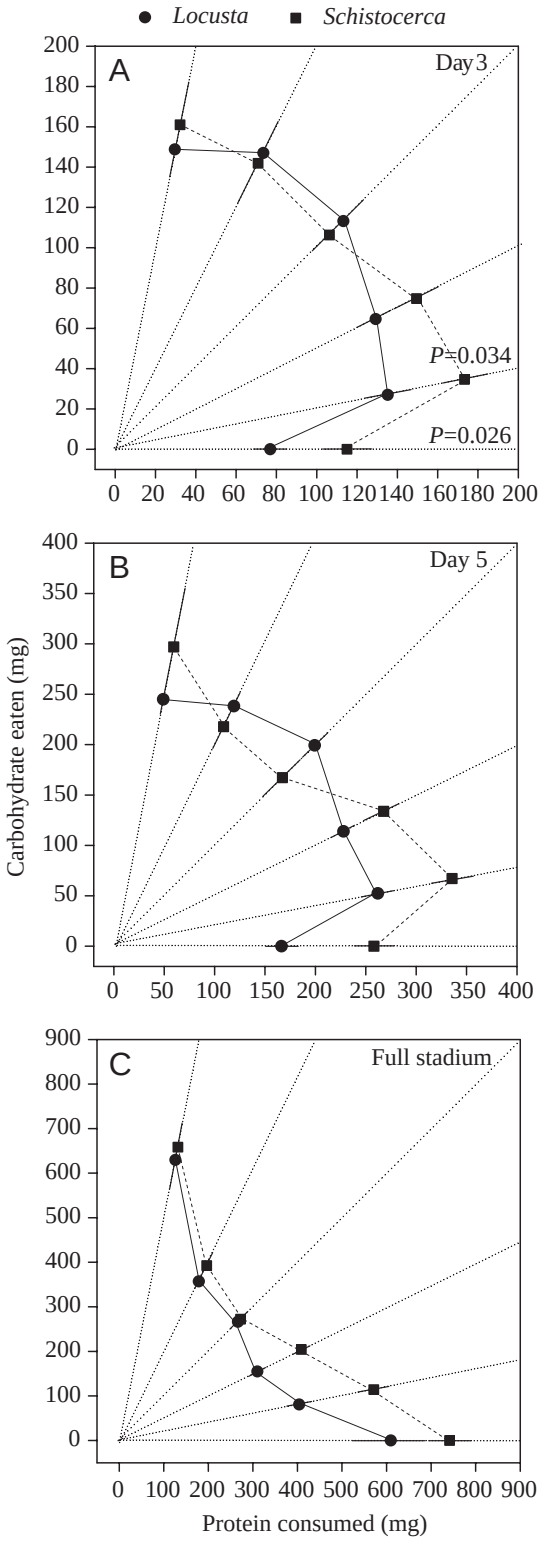

Fig. 4. Intake points of *L. migratoria* and *S. gregaria* fed one of a range of diets differing in protein:carbohydrate (P:C) ratio over the first 3 days (A) and 5 days (B) of the 5th larval stadium and across the entire stadium (C). The angle of each dotted line ('nutritional rail') depicts the P:C ratio of the food it represents. Since intake points were not free to vary other than along the relevant nutritional rail, S.E.M. are coincident with the rails (solid lines). See text for explanation of statistical tests.

data using ANCOVA with diet category as covariate, species and sex as factors and protein or carbohydrate intake as response variables. For both nutrients, the response variable was $\log_e$-transformed. The covariate was calculated as follows:

$$\log_e\left[\left(\arctan\frac{C}{P}\right)+10\right], \quad (1)$$

where C and P are the percentage of carbohydrate and protein in the foods, respectively; the term arctan C/P represents the angle (in radians) between the carbohydrate axis in Fig. 4 and the rail for each food. These transformations normalised error variances, thus justifying parametric analysis. The analysis for carbohydrate intake excluded diet 42:0, which did not contain any digestible carbohydrate, while that for protein intake included all no-choice diets.

The significant diet × species interactions in these analyses (Table 5) demonstrated that nutrient intake on excess-protein foods, but not excess-carbohydrate foods, was more strongly restricted for *Locusta* than for *Schistocerca* (Fig. 4C). The species differences were not due to differences in stadium duration, as they were also apparent for measures taken within a fixed experimental period (i.e. days 3 and 5; see above and also Fig. 4A,B). This suggests that differences in the rate of nutrient intake were involved.

Table 5. *F ratios for analysis of variance (ANOVA) on macronutrient intake across the 5th larval stadium of* Locusta migratoria *and* Schistocerca gregaria *fed one of a range of nutritionally imbalanced diets*

Dependent variable	Nutrient intake	
	Protein intake (6 diets)†	Carbohydrate intake (5 diets)†
Covariates		
Rail angle‡	877.78***	903.12***
Factors		
Species	9.616**	7.210**
Sex	4.563*	0.048
Sex × species	2.752	2.573
Factor × covariate		
Species × rail angle	8.923**	6.784**
Sex × rail angle	4.002*	0.016
Species × sex × rail angle	2.898	2.659

*, 0.05>*P*>0.01; **, 0.01>*P*>0.001; ***, *P*<0.001.

†$\log_e$ transformed.

‡Rail angle=protein:carbohydrate balance of the diet; see text for details of transformation.

Body composition

There was a significant diet × species interaction in the analysis of dry carcass mass, suggesting that the growth response of *Schistocerca* and *Locusta* to imbalanced foods differed (Table 6). From Fig. 5A it can be seen, firstly, that on excess protein foods growth was reduced in *Locusta* but not in *Schistocerca*. This effect was partly due to nitrogen (Fig. 5B) and the unaccounted constituent of body composition (Fig. 5D), but was mainly (in terms of percentage difference) due to carcass lipid content. Fig. 5C shows that for foods containing an excess of protein, carcass lipid content in *Locusta* dropped monotonically with increasing dietary protein. By contrast, in *Schistocerca*, carcass lipids stabilised at a level marginally below that observed for the self-selecting animals, and this level was maintained even on diet 42:0, which contained no digestible carbohydrates. It is worth reiterating at this point that the diets contained only trace

Table 6. *F ratios for General Linear Models on body composition and nutrient retention efficiencies for* Locusta migratoria *and* Schistocerca gregaria *fed one of a range of nutritionally imbalanced diets*

Dependent variable	Dry carcass mass†	Carcass nitrogen†		Carcass lipids‡		Unaccounted carcass mass‡	
Covariates							
Protein intake	–	–	36.868***	–	3.007	–	37.911***
Carbohydrate intake	–	–	–	–	36.611***	–	0.136
Factors							
Species	40.535***	8.015**	3.642	43.942***	2.851	38.632***	0.170
Diet	4.696***	9.989***	17.395***	49.585***	6.430***	10.264***	24.073
Sex	59.509***	59.890***	0.447	2.297	3.879	56.865***	0.001
Species × diet	4.944***	2.698*	2.556*	5.563***	3.136**	3.932	4.195**
Species × sex	1.794	3.005	1.261	0.827	4.67*	0.420	0.980
Diet × sex	1.722	1.322	2.580*	1.865	1.780	1.200	3.911**
Species × sex × diet	0.416	0.217	0.371	2.073	0.782	0.320	1.193

Hypotheses concerning retention efficiencies are tested using the residual *F* ratio for factors once the effect of nutrient intake has been taken into account as a covariate.

*, 0.05>*P*>0.01; **, 0.01>*P*>0.001; ***, *P*<0.001.

†$\log_e$ transformed.

‡Rank transformed for analysis of variance (ANOVA); untransformed for analysis of covariance (ANCOVA).

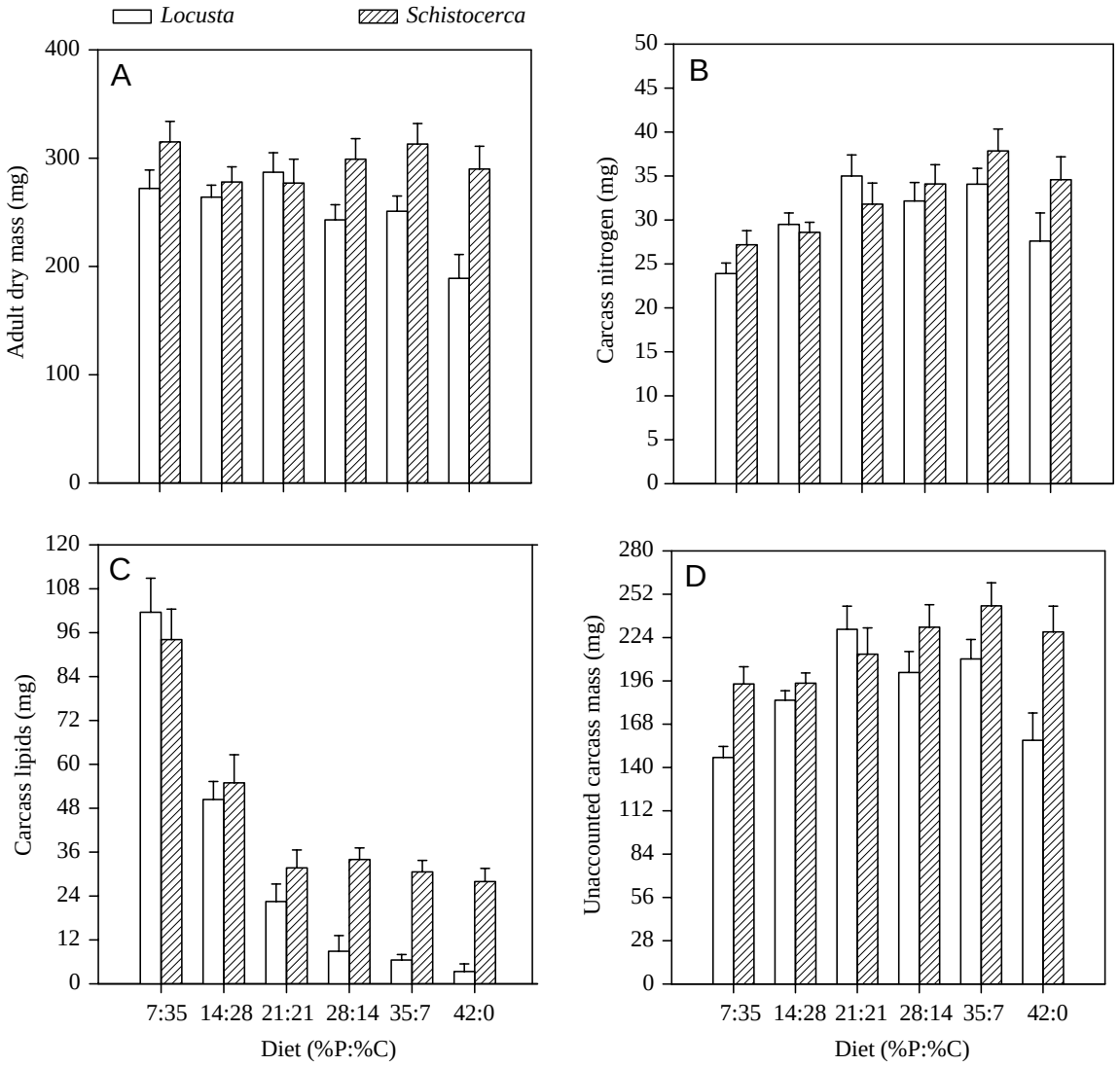

Fig. 5. Comparison of (A) adult dry mass, (B) carcass nitrogen, (C) carcass lipids and (D) unaccounted mass (carcass dry mass minus nitrogen and lipid) in *L. migratoria* and *S. gregaria* fed one of a range of diets differing in protein:carbohydrate (P:C) ratio for the duration of the 5th larval stadium.

amounts of lipid, and body lipid can therefore only have been derived from ingested carbohydrate or protein.

A second notable aspect of the pattern of carcass mass attained by the two species across foods (Fig. 5A) is that *Schistocerca* was appreciably heavier than *Locusta* on diet 7:35. This difference was not due to greater lipid stores (in fact, on this food *Locusta* had larger lipid stores than *Schistocerca*; Fig. 5C) but was apparent both for carcass nitrogen and, particularly, unaccounted body mass (Fig. 5D). The implication is that *Schistocerca* was able to allocate higher levels of protein to carcass growth than was *Locusta* when fed protein-deficient foods.

Retention efficiencies

An ANCOVA was used to test for the effects of diet on the efficiency with which ingested nitrogen was retained by the two species, with protein intake as covariate, and species, diet and sex as factors (Table 6). The fact that the diet × species interaction observed in the ANOVA on body nitrogen remained significant in the ANCOVA suggests that the pattern of nitrogen utilisation efficiencies across the diets differed between the species. The marginal means for this analysis show that *Locusta* converted ingested nitrogen with greater efficiency than did *Schistocerca* on all diets except 7:35, where the pattern was reversed (Fig. 6A).

To test whether carcass lipid content was related to differences in nutrient intake or utilisation, a model was run using protein and carbohydrate intake as covariates, and species, diet and sex as factors. A significant diet × species interaction revealed that the pattern of variation across diets differed in the efficiency with which the two species converted ingested nutrient into body lipids (Table 6). The marginal means for this analysis (Fig. 6B) show that *Schistocerca* had higher retention efficiencies on excess protein diets, while

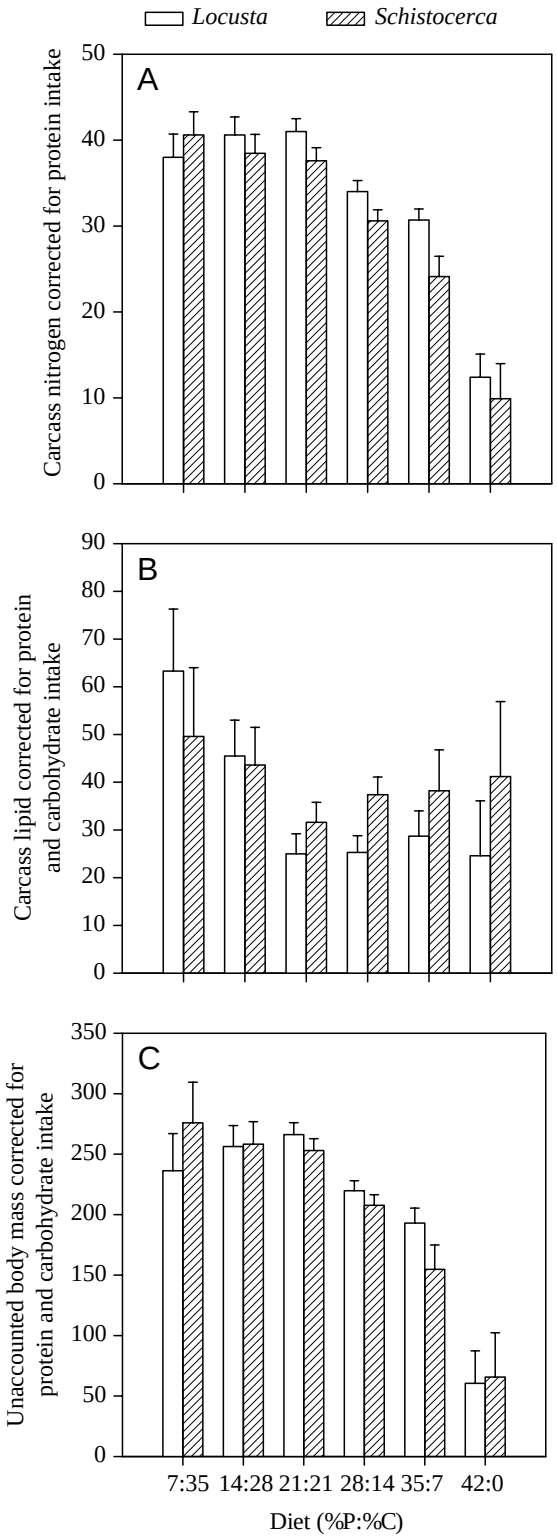

Fig. 6. Marginal means from analysis of covariance (ANCOVA; Table 6) used to estimate utilization efficiencies for *L. migratoria* and *S. gregaria* fed one of a range of diets differing in protein:carbohydrate (P:C) ratio for the duration of the 5th larval stadium. (A) Carcass nitrogen corrected for protein intake, (B) carcass lipid corrected for protein and carbohydrate intake and (C) unaccounted body mass (carcass dry mass minus nitrogen and lipid) corrected for protein and carbohydrate intake.

Locusta had higher retention efficiencies on excess carbohydrate diets. Separate analyses for the two categories of diets (excess protein and excess carbohydrate) revealed significant main effects of species (including diets 21:21, 28:14, 35:7 and 42:0, $F_{1,48}$=10.3, P=0.002; while for diets 7:35 and 14:28, $F_{1,18}$=5.0, P=0.038). This suggests that the basis for the significant diet × species interaction in the full analysis was a reversal across the species of retention efficiencies depending on which nutrient was excessive in the foods, rather than a difference on one category of diets but not the other.

The relationship between nutrient intake and the unaccounted portion of body mass was tested in an ANCOVA with protein and carbohydrate intake as covariates, and species, diet and sex as factors. As was the case in the self-selected diet, protein but not carbohydrate intake was a significant predictor of the unaccounted portion of body mass (Table 6). However, there remained a residual diet × species interaction, suggesting that the pattern across the diets in efficiency of conversion of ingested nutrients to this component of body mass differed between species. The marginal means for this effect (Fig. 6C) show that conversion efficiency was lower for *Locusta* than for *Schistocerca* on the diet containing an extreme excess of carbohydrate (7:35), was similar for the two species on diet 14:28 and then dropped off less rapidly for *Locusta* than for *Schistocerca* as the relative amount of protein in the foods increased. On diet 42:0, conversion efficiency was similar for the two species.

Discussion

The logic underlying our experiment was first to establish the preferred protein–carbohydrate intake point and the consequences of attaining this and then to measure responses to diets systematically imbalanced in relation to the preferred diet. The composition of the target food and the body composition of animals fed this food are interesting comparative data in their own right and also provide an important reference point for defining dietary imbalance and its effects within the parameters of our experimental system. We discuss selected and constrained diets in turn.

Selected diet

The pattern of nutrient selection in our two study species was significantly different, with the specialist grass-feeding *Locusta* selecting across the stadium an intake point lower in protein, and P:C ratio, than that selected by the generalist feeder *Schistocerca*. There were, however, strong suggestions that the mass-specific nitrogen requirements for growth of the two species did not differ, since there was no significant species effect on carcass nitrogen concentration (carcass nitrogen corrected for nitrogen-free body mass; Table 1). To sustain similar carcass composition in the face of lower nitrogen intake, *Locusta* adopted the complementary strategy of higher retention efficiency (significant species effect in carcass nitrogen corrected for protein intake; Table 2; Fig. 2a). Interestingly, the solitarious phase of *Schistocerca* uses ingested nitrogen more

efficiently than the gregarious phase tested here, and like *Locusta* also has a narrower host range (Simpson et al., 2002).

As was true for nitrogen, there was no difference in the mass of lipid in the carcasses of the two species; since *Schistocerca* was larger overall, this suggests that the mass-specific lipid content was lower in this species than in *Locusta*. Given that the insects in this analysis selected their own nutrient intake, and hence nutrient allocation, one interpretation of these data is that *Schistocerca* has a lower mass-specific requirement for energy storage than does *Locusta*. However, the fact that the unaccounted portion of body mass (total mass minus lipid and nitrogen) was greater in *Schistocerca* casts some doubt on this interpretation. While we did not characterise this component chemically, our analyses demonstrate that it is tightly related to protein intake, with no residual species difference (Fig. 2B; Table 2), and might well be the non-nitrogen component of ingested protein (including reduced carbon). Our data suggest, furthermore, that *Schistocerca* is capable of using ingested protein in energy metabolism since, unlike *Locusta*, this species maintained body lipid content on diets containing an excess of protein and a deficit of carbohydrate, including diet 42:0, which contained no extractable carbohydrates. Accessible energy in *Schistocerca* is therefore higher than is indicated by body lipids alone.

The data thus provide no evidence for tissue-level differences in relative nitrogen and energy requirements between a grass-specialist and a generalist that includes in its diet both grasses and forbs but do demonstrate distinct differences in their strategies for fulfilling these requirements. How can these differences be related to the nutritional characteristics of the respective evolutionary environments? There are suggestions that forbs may contain a higher proportion of nitrogen than do grasses (Mattson, 1980), and one possibility is that the higher proportion of protein in the selected diet of *Schistocerca* reflects this difference. Comparative analyses at the family and ordinal level have demonstrated that the protein:carbohydrate ratio of the target food of insects may reflect gross ecological and life-history differences such as the possession of nitrogen-upgrading symbionts (Simpson and Raubenheimer, 1993), but we are unaware of any equivalent data comparing selected intakes in taxonomically more similar forb and grass feeders.

Alternatively, the higher level of protein in the selected diet of *Schistocerca* could be an indirect consequence of having a broader host range. While a formal comparison has yet to be made, it seems reasonable to suspect that generalist feeders would encounter greater qualitative and quantitative variation in host chemistry than do specialists, and a heterogeneous diet might select for versatile ways of processing ingested nutrients. Extreme generalist cockroaches (*Periplaneta americana* L. and *Blatella germanica* L.), for example, are capable of extracting energy from refractory cellulose polymers (Mira, 1999; Jones and Raubenheimer, 2000) and also possess nitrogen-upgrading endosymbionts that enable the usual insect nitrogenous excretory product, uric acid, to be re-cycled into utilisable amino acids (Mullins and Cochran, 1986). It is interesting in this regard that *Schistocerca*, but not *Locusta*, was observed in our experiment to utilise ingested protein both as a source of nitrogen and a source of energy, this difference perhaps reflecting greater biochemical versatility of the generalist. This ability could, in turn, place a higher premium for *Schistocerca* on the acquisition of the dual-purpose protein, relative to carbohydrate, resulting in the observed protein-rich selected diet. Although the evidence that *Schistocerca* uses protein-derived carbon in energy metabolism comes from imbalanced (carbohydrate-deficient) foods, our observation that on the self-selected diet less nitrogen was retained (i.e. more was excreted) per unit of ingested protein in *Schistocerca* than in *Locusta* (Fig. 2A) suggests that the same might be true on a balanced diet.

By using a protocol in which protein and carbohydrate can be regulated orthogonally, we have therefore been able to measure the preferred intake points of protein and carbohydrate, their utilisation efficiencies and contributions to body composition in *Locusta* and *Schistocerca*. These data provide useful comparisons of the nutritional biology of the two species and generate testable hypotheses about the ecological factors that underlie the observed differences. They also provide a reference point for comparing the responses of these species to nutritionally imbalanced foods.

Imbalanced foods

A clear conclusion of our measures of intake of imbalanced and complementary foods is that, contrary to the ubiquitous assumption in optimal foraging theory (OFT; Stephens and Krebs, 1986), locusts showed no evidence of feeding in a way that maximises energy intake. According to this assumption, in the food-switching treatment, energy maximizers would feed exclusively on the high-carbohydrate food; instead, both species selected an intake point between the food rails, suggesting that the ingestive priority was to balance protein and carbohydrate intake. In the constrained diet treatments, energy prioritisation for *Locusta* would be indicated by a horizontal, linear, intake array that aligned itself with the carbohydrate co-ordinate of the intake target (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993). For *Schistocerca*, the situation is more complicated because, as our data have demonstrated, this species is capable of extracting energy both from dietary carbohydrates and proteins. In this case, the array indicating energy prioritisation would be a negatively sloped line with a gradient dependent on the relative energy densities (i.e. on the coefficient of interchangeability with respect to energy) of the two nutrient groups, a configuration similar to that observed (Fig. 4). However, the uneven performance (e.g. development times; Fig. 3) of *Schistocerca* across treatments demonstrates that, even if there was equivalence in terms of energy intake across treatments, this did not translate into functional equivalence. Both the choice and no-choice treatments in our experiment thus point to the conclusion that some currency other than energy is primary for generalist and specialist alike.

More relevant is the complex, multivariate nutritional

currency, nutrient balance. To deal with this quantitatively, a metric is needed that integrates the animal's requirements for various nutrients and its current status in relation to those requirements. Our chosen measure is 'nutritional error', which achieves an optimal value of 0 for animals that achieve their target intake and attains negative values at any point in the nutrient space that is divergent from this (Raubenheimer and Simpson, 1997, 1999). A further feature of this measure is that it is sensitive both to deficits and surpluses of the various nutrients. Given the fact that many components of the ingesta of heterotrophs are deleterious both in deficit and in surplus of some optimal rate of intake (a phenomenon that toxicologists call 'hormesis'; Gerber et al., 1999), this represents a potentially important development on the 'maximisation' assumption of OFT. We hypothesise that a primary target of selection on the nutritional biology of animals is the relative weighting that regulatory systems assign to positive (i.e. excesses) and negative (deficits) errors in the ingestion of various nutritional and non-nutritional (e.g. plant toxins; Raubenheimer, 1992; Simpson and Raubenheimer, 2001) food components.

In these terms, the intake array displayed by *Schistocerca* over days 3 and 5 indicates some coefficient of interchangeability among the *errors* in nutrient intake rather than in the value to the animal of the nutrients themselves. In the most general case, a linear intake array with negative slope shows that the ratio error P/error C is constant across nutritionally imbalanced foods, and if the linear range spans the target rail then this applies, irrespective of whether the foods contain an excess of P or C. This general case can hence be termed the 'fixed proportion' regulatory pattern. In the specific case where the slope of the line is –1, it reduces to the 'equal distance rule', where error P/error C=1 (i.e. error P = error C), which in geometrical terms means that, for a given scaling (mass in the present case), the animals feed to the point on the nutritional rail where the distance from the target in one dimension equals the distance from the target in the other dimension. The observed array for *Schistocerca* over days 3 and 5 was similar to this across all diets, with the exception of the extreme diet 42:0, where the intake point lagged behind the linear array.

The arc-shaped intake array of the grass-feeding specialist *Locusta*, by contrast, corresponds with minimising the value of (error P + error C), which in geometrical terms means feeding to the point on the nutritional rail where the value of total error incurred (i.e. across both nutrients) attains the minimum value possible for the food's composition (Simpson and Raubenheimer, 1995; Raubenheimer and Simpson, 1997, 1999). A key contrast between this, the 'closest distance rule', and the equal distance rule is that the positive errors (excesses of nutrients ingested) are greater in the latter, and so too is the total amount of nutrient ingested (Raubenheimer and Simpson, 1997, 1999; Simpson and Raubenheimer, 2000).

The patterns of regulation we observed give rise to the interesting possibility that the closest distance and equal distance rules are more broadly associated with specialist and generalist feeders, respectively (Raubenheimer and Simpson, 1997; Simpson et al., 2002). While available data are too few for a formal comparative analysis, it is suggestive that the same correspondence between host range and the pattern of nutrient balancing has been observed in the comparison of the generalist-feeding gregarious phase of *Schistocerca gregaria* and the specialist solitarious phase (Simpson et al., 2002) and also in a comparison of generalist- and specialist-feeding caterpillars (Lee et al., 2002; K. P. Lee, D. Raubenheimer, S. T. Behmer and S. J. Simpson, manuscript submitted for publication). But which selective factors might underlie the association between host range and these regulatory patterns? There is some intuitive appeal in the notion that specialist feeders, to the extent that their nutritional environment encompasses a relatively narrow range of food compositions, might forage in a manner that reduces or minimises the total nutritional error incurred. By contrast, generalist feeders encountering a wide range of food compositions might be selected for opportunistically capitalising on individual nutrients when they are encountered, even if this means temporarily diverting from a state of nutritional balance. One reason why generalists should be more robust to such diversions is that the relative breadth of their diet results in an increased probability that they will subsequently encounter a food with complementary imbalance, hence turning two excesses into useful, fitness-enhancing nutriment (Raubenheimer and Simpson, 1999; Simpson et al., 2002).

The approach that we have taken here is to attempt to correlate the patterns of macronutrient regulation with the position occupied by animals on the generalist–specialist continuum of host range. Alternatively, the pattern of macronutrient regulation might itself be used to define the nutritional strategies of animals, where an animal is considered a *nutrient* (as opposed to food plant) generalist or specialist according to the magnitude of nutritional errors (in relation to the intake target) that it tolerates. This enables us to frame the comparative question differently: to what extent does *food* generalism correspond with *nutrient* generalism? Although the studies to date show good correspondence, as mentioned in the Introduction there remains every possibility that some insects might have evolved food plant generalism as a means of reducing nutritional error; i.e. they are food generalists but nutrient specialists. Conversely, some insects with restricted host range might have evolved the capacity to tolerate wide variation in the nutrient composition of their foods. Such questions identify a need for field studies of the patterns of host plant selection by herbivores (such as that performed by Raubenheimer and Bernays, 1993), which also measure the nutritional profiles of the plants concerned and the patterns of macronutrient regulation by the animals.

Whether food specialist or generalist, it might be expected that nutrient generalists would be better physiologically adapted for dealing post-ingestively with ingested excesses than are nutrient specialists. A likely example of this from our experiments is the observation that *Schistocerca* was able to channel excess ingested protein into energy metabolism, as discussed above. Experiments using ^{13}C stable isotopes have

demonstrated that the tobacco hornworm *Manduca sexta* is similarly able to use excess ingested amino acids in energy metabolism when feeding on nutritionally imbalanced foods (Thompson, 1998). While individual hornworm larvae are host-plant specialists rather than generalist feeders – they develop induced feeding preferences for the plant on which they hatch (del Campo et al., 2001) – this species may nonetheless encounter high levels of nutritional variability since adult females lay their eggs on a range of plant species (de Boer, 1993; Mira and Bernays, 2002). The ability to use excess ingested protein in energy metabolism doubly reduces nutritional error by simultaneously decreasing the excess of ingested protein and reducing the energetic deficit due to restricted carbohydrate intake.

The capability of *Schistocerca* to deal with excess ingested protein might, on the other hand, be related to the greater nitrogen content in the selected (and ecological) diet of this species rather than its broader dietary range. In this interpretation, the response range to nitrogen of *Schistocerca* is shifted relative to *Locusta*, rather than broadened as would be expected if host range were the important factor. While possible, we consider this to be unlikely, since it would predict that *Schistocerca* should utilise protein less well than *Locusta* on foods with excess carbohydrate. In fact, *Schistocerca* showed greater nitrogen-processing efficiency on the most protein-deficient food (7:35) and was capable of maintaining body composition better than *Locusta* irrespective of the direction of nutrient imbalance. We therefore favour the interpretation that the greater concentration of protein in the selected diet of *Schistocerca* is itself a component of nutritional flexibility, providing as it does both nitrogen and energy to a physiologically flexible generalist.

In conclusion, this work has revealed some interesting differences in the patterns of nutrient balancing by a pair of generalist- and specialist-feeding grasshoppers. It is, of course, the case that dietary range is not all that differs between these species, and the observed differences could be associated with other factors. However, the fact that they were in the anticipated direction of greater behavioural and physiological flexibility in the generalists, and that similar results have been observed in independent comparisons of the patterns of intake of other generalist- and specialist-feeding insects (Simpson et al., 2002; Lee et al., 2002; Lee et al., 2002; K. P. Lee, D. Raubenheimer, S. T. Behmer and S. J. Simpson, manuscript submitted for publication), leads us to believe that macronutrient balance might have been an important selective factor in herbivorous insects. There is, at the very least, a strong case for extending this kind of analysis more broadly.

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