



Frequency-dependent food selection in locusts: a geometric analysis of the role of nutrient balancing

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We used a geometric modelling approach to investigate how changes in the frequency in the environment of foods with different nutritional profiles affect the regulation of macronutrient (protein and digestible carbohydrate) intake and the pattern of food consumption of fifth-instar nymphs of *Locusta migratoria* (L.) (Orthoptera: Acrididae). Two experiments were conducted, both with insects individually housed in arenas containing four dishes of chemically defined synthetic food. In one experiment two nutritionally imbalanced, but complementary, foods (high protein, low carbohydrate (P) and low protein, high carbohydrate (C)) were presented at different frequencies (4P:0C, 3P:1C, 2P:2C, 1P:3C and 0P:4C). The locusts regulated their feeding among the food dishes such that they defended their intake of both protein and carbohydrate, despite differences between treatments in the relative frequency of the two food types. In a second experiment, optimal (O) and suboptimal (either P or C) synthetic foods were paired in different relative frequencies. Despite the opportunity to ingest only optimal food, the locusts included suboptimal foods in their diet to varying degrees. However, they fed preferentially upon the optimal food when it was presented along with dishes of suboptimal food and they increased their fidelity to dishes of the optimal food as they decreased in frequency relative to dishes of suboptimal food. Patterns of consumption in both experiments were consistent with the control of food choices and intake being governed by a combination of known mechanisms, including direct metabolic feedback and learning.

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A general aim in the study of foraging is to develop models for predicting how animals respond to the quality, relative frequencies and distributions of potential food resources in the environment. However, the diversity of foraging processes among animals suggests that no single theoretical or experimental framework will be equally suited to all. In the study of carnivores, granivores and nectarivores, normative models predicting what an adapted animal should do under stipulated circumstances have met with success (Stephens & Krebs 1986). This is, at least partly, because for these feeding types food capture rates and handling times appear to be the primary factors limiting nutritional gain (Westoby 1974; Stephens & Krebs 1986; Bertheaux et al. 1998). Hence, realistic a priori models may be based on unitary nutrient currencies (e.g. energy) and simple choice criteria (e.g. maximization), ignoring the complexities of food composition and quality. For folivores, by contrast, such normative models may be less appropriate because the overriding criterion influencing foraging decisions appears to be nutrient

balance (Raubenheimer & Simpson 1999; Simpson & Raubenheimer 2000), and for the majority of leaf feeders too little is currently known to stipulate in advance what comprises a balanced diet and hence what it is that regulatory mechanisms have evolved to achieve (Raubenheimer & Simpson 1997).

To deal with the interactions between the uptake and use of multiple nutrients, we have developed a geometric framework for studying nutrient balancing. Briefly, this approach involves measuring the optimal proportions and amounts of various nutrients that an animal can ingest over a stipulated time period. This measure (the intake target) is then used as a reference point for assessing empirically the relative weightings that the animal assigns to the different nutrient resources in circumstances when intake is constrained by, for example, nutritionally imbalanced foods, the presence of plant toxins, unfavourable food distributions or relative frequencies (Raubenheimer & Simpson 1999; Simpson & Raubenheimer 2000). The framework differs from the normative models that have been applied in the optimal foraging literature in its emphasis on measuring, rather than assuming a priori, nutritional currencies and

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functional rules of intake. It is thus particularly well suited to animals with complex, or unknown, ingestive requirements such as herbivores.

Previous work conducted within this framework has centred on the regulatory responses of animals to foods varying in their nutrient and non-nutrient composition (Simpson et al. 1995; Raubenheimer & Simpson 1999; Simpson & Raubenheimer 1999, 2000, 2001). These studies have quantified the remarkably effective interaction of a suite of ingestive and postingestive homeostatic mechanisms for buffering the animal's performance from the vagaries of food quality. For example, locusts, *Locusta migratoria*, will strongly defend their intake of both protein and carbohydrate by selecting a diet from two suboptimal, but complementary foods (Chambers et al. 1995). They will also defend their intake of these nutrients by increasing consumption five-fold for an equivalent dilution of the diet with indigestible cellulose (Raubenheimer & Simpson 1993), and they show effective postingestive regulation when, because of the nature of the food, they are unable to achieve an optimal nutrient intake (Yang & Joern 1994a, b; Simpson et al. 1995; Raubenheimer & Simpson 1999). In addition, when confined to a single nutritionally imbalanced food, grasshoppers, *L. migratoria* and *Schistocerca gregaria*, show clearly defined patterns of trade-off between overingesting some nutrients and underingesting others, and these seem to differ according to the number of plant species eaten (Raubenheimer & Simpson 1997, 1999; Simpson & Raubenheimer 2000).

Under natural conditions a foraging folivore must deal not only with the problem of variable food quality, both within and between plants (or plant parts), but also with variation in the relative abundance within the environment of plant tissues with different nutrient composition (Laca & Demment 1996). The general question of frequency-dependent food selection in animals has received a good deal of experimental and theoretical attention, but much of this has been focused on carnivores, granivores and nectarivores (Greenwood 1984; Gendron 1987; Sherratt & Harvey 1993; Smithson & MacNair 1997). The patterns, mechanisms and functional significance of frequency-dependent food selection in animals such as folivores, whose foraging activities are complicated by feeding on nutritionally poor and variable foods, are, by contrast, little known.

We applied the geometric framework to the study of diet selection by fifth-instar locusts when the relative frequencies in the foraging environment of foods differing systematically in the balance of macronutrients were experimentally manipulated. Our experiments emulated two patterns of relative food frequencies in experimental arenas containing four dishes of chemically defined synthetic foods. First, we paired at different frequencies dishes of two nutritionally suboptimal, but complementary food types. From this experiment we were able to assess the extent to which frequency-dependent patterns of food choice subserved the requirement to obtain a balanced intake of nutrients. In a second experiment, optimal and suboptimal foods were paired at different frequencies. This experiment was designed such that any

intake of nonoptimal foods would irretrievably prevent the animals from achieving the optimal intake of the two nutrients. We were able to partition responses directly underlying the need for nutrient balancing (i.e. ingestion of the optimal food) from other causative factors in frequency-dependent food selection, such as sensory constraints and the need to gather information about alternative food types by 'sampling' (Greenwood 1984; Sherratt & Harvey 1993). In both experiments, we measured food selection over successive 2-day intervals, thus allowing us to quantify the development of selection patterns.

METHODS

Insects and Experimental Chambers

We used locusts from a culture kept under crowded conditions (i.e. in the gregarious phase state) and maintained on a diet of seedling wheat and wheat germ at the Department of Zoology, University of Oxford. Locusts are particularly appropriate subjects for a study investigating how differences in the relative abundance of foods varying in protein-carbohydrate content influence feeding. First, previous work has established that they independently regulate protein and carbohydrate intake (Simpson & Abisgold 1985; Simpson et al. 1988, 1990, 1991; Raubenheimer & Simpson 1990; Raubenheimer 1992). Second, much is known about the physiological mechanisms they use to regulate the intake of these nutrients (Simpson & Raubenheimer 1993, 2001). Finally, because locusts feed only on grass, the complication of secondary metabolites does not have to be addressed since grasses rarely contain these at biologically meaningful levels (Bernays & Barbehenn 1987).

Locusts were removed from the culture after ecdysis to the fifth stadium, weighed and then placed singly into Plexiglas arenas (23 cm in diameter and 16 cm high) which contained four dishes of synthetic food and a small plastic container (7 × 4 cm and 2 cm high with two 1.5-cm holes in the top) with water for drinking. The food dishes were modified petri dishes specifically designed to minimize spillage (Raubenheimer & Simpson 1990). Within each arena the water container was positioned centrally and the food dishes surrounded it in an equidistant and symmetrical arrangement. We also placed aluminium perches directly behind each food dish to permit the locusts to roost. Finally, partitions were placed around the outside of each arena to prevent the locusts seeing one another. All experiments were conducted in a constant temperature room at 30–32°C under a 12:12 h light:dark photoregime. Ten locusts were used in each experiment (equal numbers of males and females).

Synthetic Foods

Dry, granular chemically defined foods were made in a similar manner to those developed by Dadd (1961) and modified by Simpson & Abisgold (1985). We varied protein and digestible carbohydrate (henceforth, simply carbohydrate) to give the following three combinations of

protein (p) and carbohydrate (c): p7:c35; p19:c23; and p31:c11 (all values are expressed on a percentage dry weight basis). Previous studies had shown the p19:c23 combination to be nutritionally optimal for fifth-instar *L. migratoria* (Chambers et al. 1995); the other two foods are suboptimal mixtures that are symmetrically imbalanced on either side of the optimal p19:c23 food. All three foods had equal total amounts of protein plus carbohydrate and, therefore, also contained identical proportions of the other ingredients, including indigestible cellulose powder. Stadium duration on the optimal food (p19:c23) is typically 10–11 days at a temperature of 30°C (e.g. Raubenheimer & Simpson 1993).

Experimental Protocol

We did two experiments. In the first, dishes of suboptimal food, p31:c11 (P) and p7:c35 (C), were paired together in different frequencies, ranging from all P dishes to all C dishes, to give five combinations: (1) 0P:4C, (2) 1P:3C, (3) 2P:2C, (4) 3P:1C and (5) 4P:0C. In the second experiment, optimal food, p19:c23 (O), was paired with one of the two suboptimal foods (P or C) in different frequencies. Here treatments ranged from all dishes containing optimal food to all dishes containing suboptimal food. This resulted in nine combinations: (1) all C, (2) 1O:3C, (3) 2O:2C, (4) 3O:1C, (5) all O, (6) 3O:1P, (7) 2O:2P, (8) 1O:3P and (9) all P.

In both experiments identical protocols were followed. First, to assign treatments to individual arenas we used a completely randomized block design. Next, each dish within an arena received a number (1–4). This allowed us to identify the nature of the contents of individual dishes and to return dishes, after they had been removed and weighed, to the same location in the arena. We weighed each dish to the nearest 0.1 mg after allocating it to its synthetic food and allowing the food to equilibrate to ambient room humidity levels (RH 30–40%) for ca. 24 h. The locusts were then allowed to feed for 48 h, after which we removed each dish within an arena and replaced it with a fresh, preweighed dish of food. The food dish that we removed was allowed to equilibrate to room humidity levels before it was reweighed. We repeated this protocol at the end of day 4. Day 6 was the last day of the experiment, after which we removed and weighed the locusts. Again we allowed the food in the arenas to equilibrate to room temperature before weighing it.

Statistical Analysis

To analyse various aspects of food consumption, as well as the weight gained by the locusts, we used ANOVA, analysis of covariance (ANCOVA) and multivariate analysis of covariance (MANCOVA) with the statistical packages SAS 6.12 (SAS Institute 1990) and SuperANOVA (Gagnon et al. 1991). For covariate analyses, we used the locusts' initial mass to correct for size differences (e.g. females are larger than males). When necessary, the data were log transformed to meet the underlying assumptions of these analyses. When significant differences

between treatments were detected, pairwise comparisons were made with post hoc comparisons and/or contrasts. Contrasts for MANOVAs followed the techniques used by Scheiner (1993). Where multiple contrasts were made, α levels were adjusted with Bonferroni methods.

RESULTS

Experiment 1: Paired Complementary Suboptimal Foods

Food selection

Despite differences in the relative frequency of the two food types between treatments (e.g. 1P:3C, 2P:2C and 3P:1C), the amount of p31:c11 food eaten over the entire experiment (days 0–6) was similar (Fig. 1a, Table 1). Similarly, the total amount of p7:c35 food eaten across treatments was similar. This meant that on a per dish basis, the amount eaten of a given food type increased as the relative frequency of that food type decreased.

To examine the time course of food selection patterns, we also analysed consumption patterns for each time interval. As seen during days 0–6, no difference in consumption of the two respective food types was observed across treatments during days 2–4 and 4–6, respectively (Fig. 1c, d, Table 1). This again indicates that on a per dish basis fidelity to a given food type increased as it became less frequent in the arenas. Over the first 2 days of the experiment, however, there was a significant between-treatment difference in relative consumption of the two food types, such that on the 1P:3C treatment less p31:c11 food and more p7:c35 food was eaten compared to the 3P:1C treatment (Fig. 1, Table 1). None the less, as was observed over subsequent time intervals, during days 0–2 the amount of food eaten on a per dish basis increased as the frequency of a given food type decreased. In addition, within a treatment the amounts of the different food types eaten, relative to each other, changed with time. During the first 4 days more p31:c11 food was eaten but during days 4–6 similar amounts of p31:c11 and p7:c35 food were eaten.

Nutrient intake

Figure 2 shows a bicoordinate plot of the amounts of protein and carbohydrate eaten over the entire experiment (days 0–6) and for each of the different 2-day intervals. Over the entire experiment and during days 2–4 and 4–6, locusts regulated their protein–carbohydrate intake to statistically identical points when they were on treatments with at least one dish of each food type (Table 2). It was only during days 0–2 that differences between treatments were observed, with locusts on the 3P:1C treatment failing to match the protein–carbohydrate intake of locusts reared on the 1P:3C and 2P:2C treatments (Table 2). Locusts on the 0P:4C and 4P:0C treatments, by contrast, were confined to fixed nutritional 'rails' and thus constrained to achieve intake points different from each other and from the selected intake point of the other treatments.

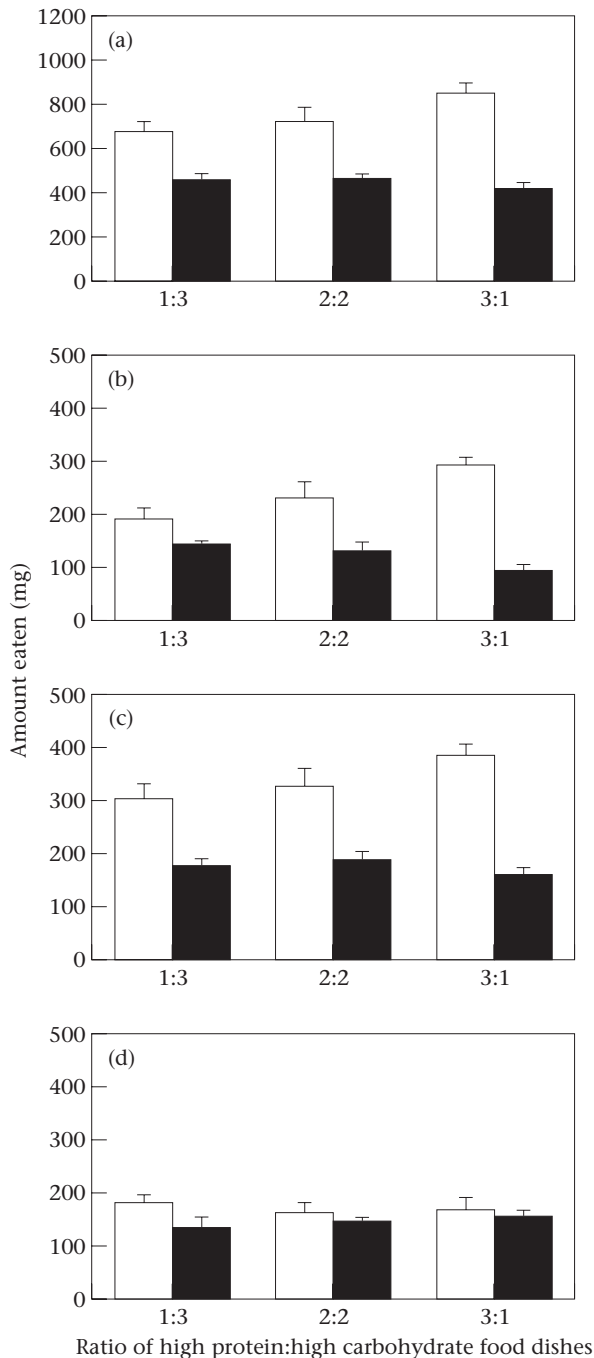


Figure 1. The average \pm SE total amount of the high carbohydrate (■; p7:c35) and high protein (□; p31:c11) foods eaten during the first experiment. Only treatments with both food types are shown. (a) Days 0–6, (b) days 0–2, (c) days 2–4 and (d) days 4–6.

Total intake and growth

Total food intake was similar across the majority of the treatments but a significant treatment effect was observed because on the OP:4C treatment intake was low (Fig. 3, Table 3). Mass gained was also similar across the different treatments with the exception of the OP:4C treatment (Fig. 3), which was significantly lower than the other treatments (Table 3). For both of the previously

mentioned analyses, the effect of sex and the treatment-by-sex interaction were not significant. Initial mass was significant as a covariate for total intake but not for mass gain.

Experiment 2: Paired Optimal and Suboptimal Foods

Food selection

Over the entire experiment (days 0–6), the amount of the optimal food eaten on the 1O:3S treatment was significantly lower than on the 3O:1S and 2O:2S treatments (Fig. 4, Table 4). Similarly, the amounts of suboptimal foods eaten on the 1O:3S treatments were greater than on treatments with one or two dishes of suboptimal food. In contrast, there were no significant differences in the amounts of optimal and suboptimal food types eaten on the 3O:1S and 2O:2S treatments (Fig. 4, Table 4). When consumption is expressed on a per dish basis, however, the average amount of optimal food eaten increased as the number of dishes in the arenas containing optimal food decreased.

Comparison of the patterns of food consumption over separate time intervals indicated that during days 2–4 and 4–6 the amounts of optimal and suboptimal food eaten were similar across treatments (Table 4). Expressed on a per dish basis, however, the consumption of optimal food increased significantly as the number of optimal food dishes in an arena decreased. By contrast with later periods, over days 0–2 differences in the total amount of optimal and suboptimal food eaten were observed for each paired comparison (Table 4). The amount of optimal food consumed on a per dish basis did, none the less, increase as the number of these food dishes decreased. Additionally, for days 0–2 and 2–4, more p31:c11 food was eaten, compared with p7:c35 food (Table 4); no difference in the consumption of the different suboptimal food types was observed, however, during days 4–6. In none of the analyses were there significant main effects of sex or significant interactions between food type and the ratio of optimal to suboptimal food.

Nutrient intake

Figure 5 shows a bicoordinate plot of the amounts of protein and carbohydrate eaten over the entire experiment (days 0–6) and for each of the 2-day intervals. Here the treatments of greatest interest are those pairing optimal food with suboptimal food (either P or C) at different frequencies. Over the entire experiment the protein-carbohydrate intake of locusts from treatments with at least one dish of each food type did not overlap with that from the treatment where all dishes contained optimal food. This is because locusts ate from all the dishes and the intake of suboptimal food irretrievably pulled insects away from the optimal food rail. Second, irrespective of whether the suboptimal food was protein- or carbohydrate-deficient, the degree to which nutrient intake diverged from the optimal food was relatively independent of the frequency of imbalanced food dishes in the environment (Fig. 5, Table 5). For example, no

Table 1. MANCOVAs for food consumption from the first experiment

Source	df Hypothesis, Error	F values			
		Days 0–6	Days 0–2	Days 2–4	Days 4–6
Amounts consumed from the different food dishes					
Treatment	4,44	1.49	3.25*	1.25	1.51
Sex	2,21	2.00	0.84	1.98	0.57
Treatment×Sex	4,44	1.02	0.78	0.59	1.74
Initial mass	2,21	0.88	0.57	1.66	2.09
Multivariate contrasts (*significant difference w/ $\alpha=0.05/3$)					
1P:3C versus 2P:2C	2,21	—	1.10	—	—
1P:3C versus 3P:1C	2,21	—	7.61*	—	—
2P:2C versus 3P:1C	2,21	—	0.57	—	—

F values (Pillai's trace) are shown for the entire experiment (days 0–6) and for each time interval. The analysis was restricted to treatments that contained both food types (three in total: 1P:3C, 2P:2C and 3P:1C; P is high protein and C high carbohydrate food). Initial mass was used as a covariate to adjust for size differences between insects. * $P < 0.05$. F values for multivariate contrasts between the treatments containing both C and P foods are also reported (contrasts were not performed if the treatment effect was not significant).

significant difference in protein–carbohydrate intake was observed between the 2O:2C and 3O:1C treatments, or between the 2O:2P and 3O:1P treatments. Significant differences did exist between these and the more extreme treatments (1O:3C and 1O:3P), however.

Protein–carbohydrate intake on the different treatments was also tracked over days 0–2, 2–4 and 4–6. More significant differences were observed when a carbohydrate-deficient (P) food was paired with O food at different frequencies than when protein-deficient (C) food was paired with O food. In addition, the greatest number of observed differences was recorded during the

first period (days 0–2), with four of the six comparisons showing significant differences (Table 5). During each of the later two periods (days 2–4 and 4–6) only two of the six comparisons made were considered significantly different from one another (Table 5).

Total consumption and growth

In this experiment the total amount of food eaten was significantly affected by the starting weight of the locusts (larger locusts ate more than smaller locusts) but no main effects or interactions were significant (Table 6, Fig. 6). When mass gained was analysed, however, a significant interaction between food type and the ratio of optimal to suboptimal food was found (Table 6, Fig. 6). As the number of C food dishes in the arenas increased, weight gain decreased (one-tailed linear contrast: $F_1=4.09$, $P=0.025$). No change in mass gain was observed as the number of P dishes in the arenas increased (one-tailed linear contrast: $F_1=0.05$, $P=0.820$).

DISCUSSION

It is well known that animals consuming a number of different food types may vary their relative preference for these according to the densities of each food type in the foraging environment. This phenomenon, known as frequency-dependent selection, has best been studied in carnivores, most commonly focusing on the consequences of predator foraging behaviour for the relative fitness of rare versus common prey types (or, in the case of nectarivores, flower morphs, e.g. [Smithson & MacNair 1997](#)). Hence, cases where a predator eats disproportionately more of a common food item have been termed 'proapostatic selection', to reflect the fact that rare forms are at a selective advantage. Conversely, predator preference for the rarer form has been termed 'antiapostatic selection' ([Greenwood 1984](#)).

Our experiments are unusual in that they focus on frequency-dependent food selection in herbivores (but see [Chandra & Williams 1983](#); [Cottam 1985](#); [Pennings](#)

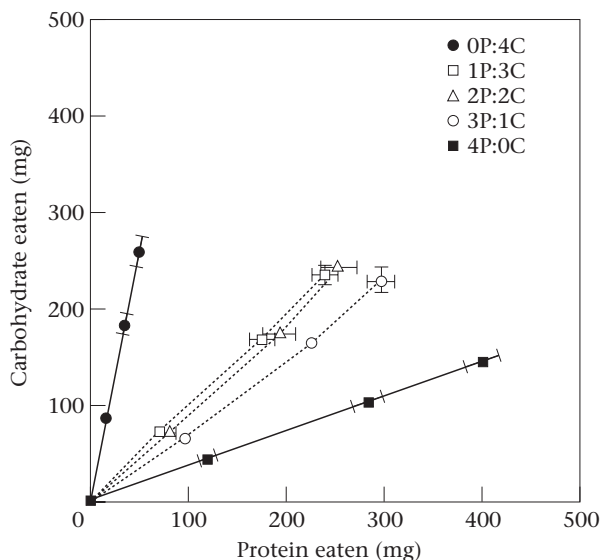


Figure 2. Bivariate means+SE for protein (P) and carbohydrate (C) intake for locusts given four dishes of p7:c35 (C) food, four dishes of p31:c11 (P) food, or combinations of C and P foods at different frequencies, during the first experiment. —: 'Rails' for the single food type treatments; · · ·: rails for the treatments containing at least one dish of each food type. The first, second and third symbol along each rail indicates the intake point reached after 2, 4 and 6 days, respectively.

Table 2. MANCOVAs for protein and carbohydrate intake from the first experiment

Source	df Hypothesis, Error	F values			
		Days 0–6	Days 0–2	Days 2–4	Days 4–6
Protein and carbohydrate intake					
Treatment	8,76	52.55**	20.57**	30.73**	18.03**
Sex	2,37	0.28	0.60	0.43	1.74
Treatment×Sex	8,76	1.07	1.27	0.38	1.45
Initial mass	2,37	5.91*	0.99	4.90*	2.65
Multivariate contrasts (*significant difference w/ $\alpha=0.05/3$)					
1P:3C versus 2P:2C	2,37	0.05	0.39	0.03	2.48
1P:3C versus 3P:1C	2,37	3.74	8.05*	2.76	4.37
2P:2C versus 3P:1C	2,37	4.33	4.84*	2.91	0.31

F values (Pillai's trace) are shown for the entire experiment (days 0–6) and for each time interval. Treatment refers to the different combinations of p7:c35 and p31:c11 foods (five in total; P refers to protein and C to carbohydrate). Initial mass was used as a covariate to adjust for size differences between insects. * $P<0.05$, ** $P<0.01$. F values for multivariate contrasts between the treatments containing both high carbohydrate (C) and high protein (P) foods are also reported.

et al. 1993) and, as far as we know, are unprecedented in that they were designed to test functional, rather than ecological or population-genetic consequences. Furthermore, the considerable amount that is known about physiological and behavioural aspects of locust nutrition (reviewed by Simpson et al. 1995; Simpson & Raubenheimer 2000) means that our data are

readily interpretable in relation to the underlying mechanisms.

Results from our first experiment showed that, where locusts had access to two complementary, suboptimal foods, they distributed their feeding between food dishes according to the relative frequency of each food type, thus showing frequency-dependent selection. Since on a per dish basis this involved increased feeding upon the rarer food type, it provides an additional case of antiapostatic selection in herbivores. Chandra & Williams (1983) also found that *Schistocerca gregaria* grazed selectively on less-favoured plants when they were relatively less abundant in an experimental arena. Similarly, Pennings et al. (1993) showed that the sea hare, *Dolabella auricularia* (Gastropoda: Opisthobranchia), increasingly preferred the rarer of two algal species as its relative abundance decreased. By contrast with the few existing studies on herbivores, carnivores have most frequently been found to show proapostatic selection, in which commoner

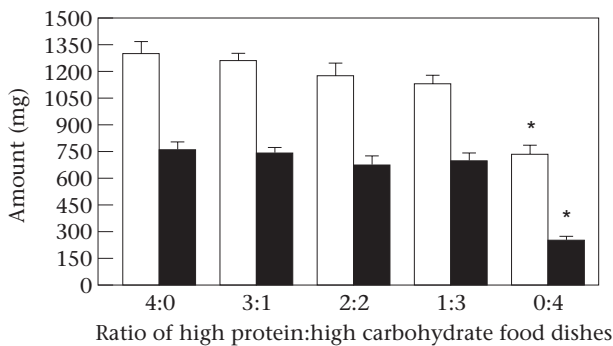


Figure 3. The average+SE total dry mass of food eaten (□) and wet mass gained (■) during the first experiment. An asterisk above a bar indicates a significant difference from the 2P:2C treatment (Dunnett's test with $\alpha=0.05$).

Table 3. ANCOVAs comparing the total amount of food consumed and mass gained over the first 6 days of the first experiment

Source	df	F value	
		Total consumption	Mass gain
Treatment	4	40.15**	39.61**
Sex	1	1.62	1.20
Treatment×Sex	4	1.28	1.72
Initial mass	1	11.12**	2.28
Error	38,37		

Initial mass was used as a covariate to adjust for size differences between insects. ** $P<0.01$.

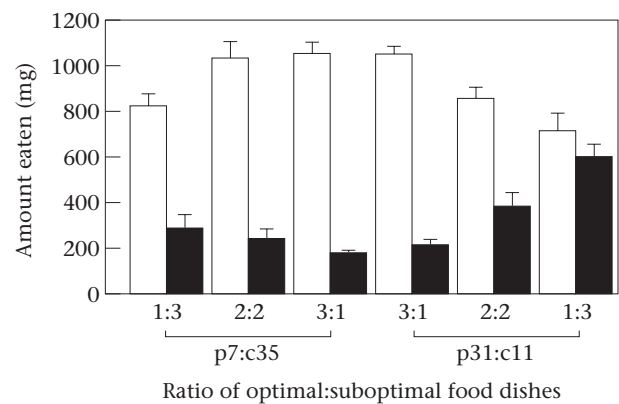


Figure 4. The average+SE total amount of optimal (□) and suboptimal (■) food eaten over days 0–6 during the second experiment. The optimal food was p19:c23, where p refers to protein and c to carbohydrate. The two suboptimal foods were high carbohydrate (p7:c35) and high protein (p31:c11). Consumption patterns for days 0–2, 2–4 and 4–6 did not differ appreciably from that during days 0–6 and are therefore not shown.

Table 4. MANCOVAs for food consumption from the second experiment

Source	df Hypothesis, Error	F values			
		Days 0–6	Days 0–2	Days 2–4	Days 4–6
Amounts consumed from the different food dishes					
Type	2,47	8.51**	4.01*	7.27*	3.03
Ratio	4,96	6.42**	10.96**	2.34	1.91
Sex	2,47	1.06	2.24	1.30	1.63
Type×Ratio	4,96	1.00	1.88	0.81	1.08
Initial mass	2,47	5.98**	3.78*	4.19*	0.33
Multivariate contrasts (*significant difference w/ $\alpha=0.05/3$)					
1O:3S versus 2O:2S	2,47	7.05*	8.31*	—	—
1O:3S versus 3O:1S	2,47	16.49*	35.18*	—	—
2O:2S versus 3O:1S	2,47	2.07	10.41*	—	—

F values (Pillai's trace) are shown for the entire experiment (days 0–6) and for each time interval. Type refers to whether the optimal (O) food was paired with p7:c35 (C) or p31:c11 (P) food (where p refers to protein and c to carbohydrate), and Ratio is the number of optimal to suboptimal (S) food dishes in an arena (3O:1S, 2O:2S or 3O:1S). Initial mass was used as a covariate to adjust for size differences between insects. * $P<0.05$, ** $P<0.01$. F values for multivariate contrasts between the treatments containing different ratios of O and S foods are also reported (contrasts were not performed if the ratio effect was not significant).

forms of prey are favoured (Allen 1988; Sherratt & Harvey 1993).

Why this difference between carnivores and herbivores? Most explanations proposed for proapostatic selection in carnivores invoke advantages in detecting and locating common, compared to rarer, prey types (Greenwood 1984; Sherratt & Harvey 1993). Conversely antiapostatic selection might be expected when

rare forms are easier to detect than common forms (Greenwood 1984). However, on the scale of experimental arenas used in most laboratory studies on herbivorous insects (ours being no exception), food location is unlikely to be an issue. Perhaps more relevant is Greenwood's (1984) prediction that antiapostatic selection should be common where there are nutritional reasons for benefiting from a mixed diet. For example, Pennings et al. (1993) hypothesized that nutrient regulation is the most likely explanation for why the rarer of two algal species became preferred by sea hares as its relative abundance decreased. This explanation has since acquired additional indirect support, as internal nutritional status has recently been strongly implicated in food selection in another generalist gastropod, the field slug, *Deroceras reticulatum* (Cook et al. 2000). Another benefit of a mixed diet is dilution of intake of deleterious compounds (Freeland & Janzen 1974). Chandra & Williams (1983) invoked a need to avoid ingesting high concentrations of any single plant toxin as the primary functional reason for *S. gregaria* favouring rare plants.

In the present study we quantified the nutritional outcomes of the observed distribution of feeding across available food dishes, which provided direct evidence for antiapostatic selection subserving the need to ingest a balanced diet. In the treatments containing both food types, locusts regulated their intake such that they defended the balance and amounts of macronutrients ingested. This strongly suggests that the defended intake point is a functional optimum (Simpson & Raubenheimer 1995; Raubenheimer & Simpson 1997), an interpretation corroborated by the fact that locusts on treatments with both food types, regardless of the frequency with which these foods were paired, achieved similar growth during the experiment.

By contrast with the first experiment, where locusts had to mix their intake appropriately from two food types to obtain a balanced diet, in the second experiment a

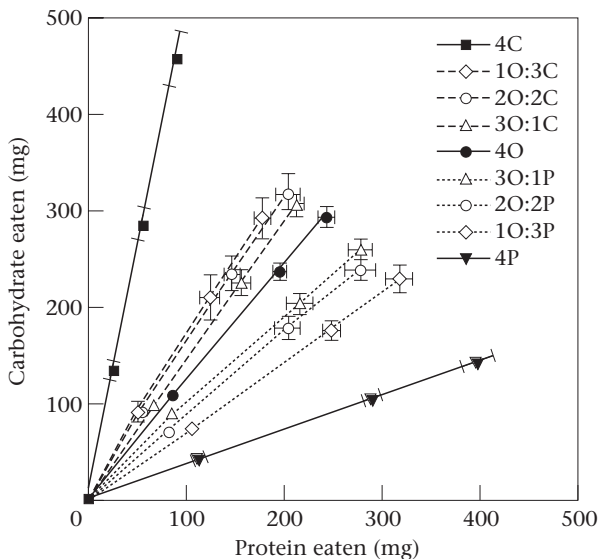


Figure 5. Bivariate means+SE for protein (P) and carbohydrate (C) intake for locusts given four dishes of p7:c35 (C) food, four dishes of p19:c23 (O) food, four dishes of p31:c11 (P) food, or combinations of O food with either C or P foods at different frequencies during the second experiment. —: 'Rails' for the single food type treatments; ---: rails where at least one O food is paired with C food; ···: rails where at least one O food is paired with P food. The first, second and third symbol along each rail indicates the intake point reached after 2, 4 and 6 days, respectively.

Table 5. MANCOVAs for protein and carbohydrate intake from the second experiment

Source	df Hypothesis, Error	F values			
		Days 0–6	Days 0–2	Days 2–4	Days 4–6
Protein and carbohydrate intake					
Treatment	16,144	18.19**	18.64**	14.64**	12.36**
Sex	2,71	1.50	1.49	0.87	1.13
Treatment×Sex	16,144	1.75*	1.09	1.21	0.97
Initial mass	2,71	14.80**	4.39*	3.24*	1.95
Multivariate contrasts (*significant difference w/ $\alpha=0.05/6$)					
p19:c23 (O) paired with p7:c35 (C)					
1O:3C versus 2O:2C	2,71	6.05*	3.37	4.67	2.79
1O:3C versus 3O:1C	2,71	7.38*	13.83*	2.88	2.53
2O:2C versus 3O:1C	2,71	0.10	4.39	0.40	0.27
p19:c23 (O) paired with p31:c11 (P)					
1O:3P versus 2O:2P	2,71	8.17*	17.14*	7.28*	2.05
1O:3P versus 3O:1P	2,71	19.07*	36.99*	11.08*	8.61*
2O:2P versus 3O:1P	2,71	3.20	5.93*	0.64	5.60*

F values (Pillai's trace) are shown for the entire experiment (days 0–6) and for each time interval. Treatment refers to the different combinations of optimal and suboptimal foods (see Table 4 for details). Initial mass was used as a covariate to adjust for size differences between insects. * $P<0.05$, ** $P<0.01$. F values for multivariate contrasts between the treatments containing different ratios of O to C food and different ratios of O to P foods, respectively, are also reported.

balanced diet could be acquired only through avoiding mixing, and feeding entirely from the balanced food. This design enabled us to detect residual tendencies to mix foods once the need for nutrient balancing had been factored out, and to quantify the effects on this of relative frequency in the environment of balanced and imbalanced foods. Locusts were not completely faithful to the nutritionally balanced food when suboptimal foods were also present in the arena. As a consequence, these locusts were irretrievably diverted from achieving their intake target (Fig. 5). However, the extent to which nutrient intake differed from optimal was relatively independent of the frequency of suboptimal food dishes in the environment, with significant differences existing only on the extreme (1O:3C and 1O:3P) treatments. Locusts did this by increasing per dish preference for the balanced food as it became rarer in the environment, again suggesting that nutrient balancing underlies antiapostatic

selection in these animals. By contrast, the per dish preference for the alternative, suboptimal food remained relatively constant irrespective of its frequency in the environment (Fig. 4).

The frequency-independent selection of suboptimal foods provides some general pointers to the kinds of phenomena involved. Specifically, it suggests the involvement of underlying processes that are less tightly homeostatic than was observed when locusts fed on complementary foods (the first experiment) or on the optimal food in the second experiment. One possibility is that the sensory capabilities of our locusts constrain the extent to which they can reliably distinguish between the foods, resulting in food selection errors (see models

Table 6. ANCOVAs comparing the total amount of food consumed and mass gained over the first 6 days of the second experiment

Source	df	F value	
		Total consumption	Mass gain
Type	1	0.00	18.70**
Ratio	4	0.62	5.55**
Sex	1	0.03	2.88
Type×Ratio	8	1.38	9.87**
Initial mass	1	23.57**	10.00**
Error	38,37		

Initial mass was used as a covariate to adjust for size differences between insects.

** $P<0.01$.

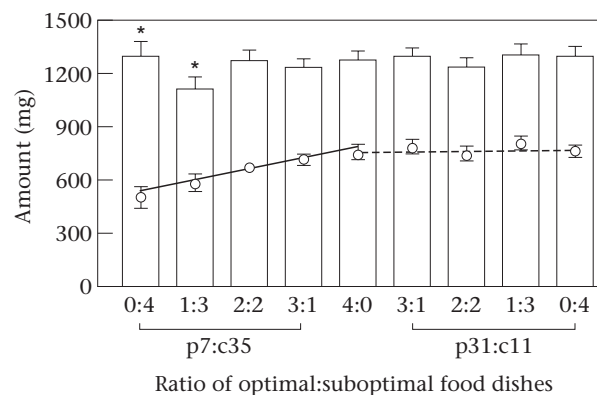


Figure 6. The average \pm SE total dry mass of food eaten (\square) and wet mass gained (\circ) during the second experiment. An asterisk above a weight gain symbol indicates a significant difference relative to the all O treatment (Dunnett's test ($\alpha=0.05$)). Linear responses to changes in the number of C food dishes (—) and P food dishes (---) are shown. See text for details.

in Simpson & Raubenheimer 1996). Alternatively, as discussed by Greenwood (1984), sampling a variety of foods might be an adaptive behaviour, assisting animals to assess the value of novel food items (see Day et al. 1998 for an extreme stance on this issue). A third possibility is that animals are adapted to switch 'compulsively', a strategy that would lead to ingestion of a nutritionally balanced diet only in an environment with a mean composition across foods that represents an animal's optimal requirements (Bernays et al. 1992; Raubenheimer & Bernays 1993). Our data do not discriminate between the first two of these hypotheses, as both would predict that the ingestion of imbalanced foods would increase with their density in the environment. However, we can discount the third in the present case because of the patently nonrandom, homeostatic selection behaviour.

Whatever the detailed explanation, there are indications that selection pressures countering the inclusion in the diet of nutritionally imbalanced foods at low levels might be weak at best. For instance, in our experiment, despite the differences between treatments in protein-carbohydrate intake, differences in mass gain were minimal. This suggests that locusts unable to reach the intake targets via food mixing use postingestive mechanisms to ameliorate the impact of suboptimal ingested diets, as seen in previous experiments (Zanotto et al. 1993, 1994, 1997). Furthermore, in many instances in nature an animal that deviates towards an excess of one nutrient is likely to encounter a complementary food that redresses the imbalance. As the first experiment showed, locusts are capable of selectively feeding on complementary foods.

A further notable pattern in Fig. 5 is that the extent to which the locusts deviated from the optimal intake was greater when the imbalanced food contained excess protein, than excess carbohydrate. This was most likely an artefact of our estimation of the composition of the target food. Based on previous work (Chambers et al. 1995), a 19% protein and 23% digestible carbohydrate (a ratio of 1:1.2) mixture was found to be optimal. In contrast to this earlier work, locusts in the first experiment selected a protein-carbohydrate ratio of 1.2:1, which lies symmetrically between the two groups of selected intake points in Fig. 5. The discrepancy in composition of the optimal food between the present and earlier experiments may reflect the somewhat higher temperature under which the current experiment was conducted than in the earlier work. That temperature differences might have been important is further indicated by the faster development rate of the locusts under the present conditions (8–9 days at 30–32°C versus 10–11 days at 29–31°C). A further between-experiment difference that deserves mention is how in the first experiment total consumption of excess carbohydrate foods in the 4C:0P treatment was low compared with the same treatment in the second experiment. It is not clear why this occurred, but it is known that the p7:c35 food is close to being unsatisfactory as a diet, lying on the threshold of suitability for survival (Raubenheimer & Simpson 1993; Simpson & Raubenheimer 1995).

Food mixing is a prominent feature of foraging in insects, which has been observed several times in field studies (Bernays et al. 1992; Raubenheimer & Bernays 1993; Howard et al. 1994; Chambers et al. 1996). Although few data currently exist, it seems probable that diet mixing is partly due to the interspersed of nutritionally balanced, imbalanced and complementary foods, as emulated in the present experiments. If this is the case, the mechanisms insects use to defend their protein-carbohydrate intake target will involve an interaction between the haemolymph (blood) and mouthpart taste receptors (Simpson et al. 1995). How such an interaction could explain patterns of consumption in our study can be seen with the 3C:1P treatment. After a meal on the C food, haemolymph sugar titres would have been high because the amount of digestible carbohydrates present in the C food would have exceeded the demands for growth and metabolism (Zanotto et al. 1996). In contrast, amino acid titres would have been low. Since insects experiencing nutrient deficits tend to locomote, movement in search of an alternative food would have been induced (Cohen et al. 1987; Naeem et al. 1992). If the next dish encountered contained C food, it would have been rejected or only a small meal taken since the chemostimulatory inputs required to sustain feeding would have been low because of the high haemolymph sugar titres incurred from the previous meal. Upon encountering the dish of P food, however, the combination of low amino acid titres in the haemolymph and high concentrations of amino acids present in the food would have provided the necessary stimulatory input to initiate and sustain feeding (Simpson & Simpson 1992). For locusts on the 1C:3P treatment an opposite reaction to sugar and amino acids in the food would have been expected.

Finally, in addition to direct, blood-borne feedbacks, grasshoppers use learning to regulate nutrient intake (Simpson & White 1990; Raubenheimer & Tucker 1997; Behmer et al. 1999; Dukas & Bernays 2000). Our results suggest that learning might have been occurring in both experiments. Differences in patterns of food consumption were observed between treatments during the early part of the study (days 0–2), but thereafter locusts improved in their ability either to mix complementary foods (the first experiment) or to feed preferentially on the optimal food (the second experiment).

Our data have further shown the remarkable capabilities of locusts to regulate their nutrient intake, in this case in the face of varying relative frequencies of food types of differing nutritional composition in their environment. More generally, the experiments provide direct evidence supporting nutrient regulation as a functional explanation for frequency-dependent food selection in herbivores.

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