



Food distance and its effect on nutrient balancing in a mobile insect herbivore

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We investigated how the distance between foods of differing nutrient content affects macronutrient (protein and digestible carbohydrate) regulation and the patterns of food acquisition, movement and feeding activity in fifth-instar nymphs of *Locusta migratoria* (L.) (Orthoptera: Acrididae). We placed individual insects into one of three differently sized circular arenas (20, 40 or 80 cm diameter) that contained four dishes of chemically defined synthetic food. One of these dishes contained high-protein, low-carbohydrate food (P), and the other three dishes contained low-protein, high-carbohydrate food (C). Alone, these foods are nutritionally unbalanced, but together they are complementary. Regardless of arena size, locusts regulated their protein–carbohydrate intake to similar points, and in all three arenas they ate preferentially from the dish containing the P-food. We also recorded the patterns of foraging behaviour for 12 h on days 1 and 4 of the experiment, which allowed us to determine the effect of distance between foods and whether behaviour was modified with experience in the different arenas. Locusts' foraging behaviour in small arenas was similar on days 1 and 4, and the time they spent in different parts of the arena did not differ from that predicted by the simplest random probability model. In contrast, the foraging behaviour of locusts in the medium and large arenas changed between days 1 and 4, including spending more time in the area of the arena containing the P-food and visiting fewer of the C-food dishes. We discuss how distance between foods influences foraging strategies, and the possible role of learning in its development.

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The distance separating foods influences foraging behaviour in animals (Charnov 1976; Stephens & Krebs 1986), including insect herbivores (e.g. Kareiva 1982; Cain 1985; Bernays et al. 1997), but how it interacts with the need to regulate intake of multiple nutrients is unclear. Laboratory studies show that insect herbivores are adept at regulating nutrient intake (e.g. Cohen et al. 1987a, b; Raubenheimer 1992; Chambers et al. 1995). In nature, they can attempt to do this by feeding on different plants or plant parts (Schultz 1983; Raubenheimer & Bernays 1993; Chambers et al. 1996). However, as the distance between nutritionally complementary food resources increases, the demands on the regulatory systems and the costs associated with regulating nutrient intake, such as increased energy expenditure and predation risks (Lima & Dill 1990; Abrams 1993; Sih 1993), are likely to increase. It might thus be expected that insect herbivores in habitats where complementary food items are close together (relative to the size and mobility of the animal) would regulate their nutrient intake more tightly than

herbivores in habitats where complementary foods are further apart. Alternatively, the impact of increased distance between complementary foods on nutritional regulation may be ameliorated by herbivores adjusting their foraging strategies. For example, as the distance between complementary foods increases, herbivores might increase fidelity to rare food types, decrease levels of activity or increase foraging efficiency by taking more directed feeding excursions.

For insect herbivores, protein and carbohydrates are the most strongly regulated nutrient groups, and as a result these two nutrient groups have been the focus of the majority of studies investigating issues related to nutrient regulation. To date, the African migratory locust, *Locusta migratoria* L., has been the most intensively studied herbivore, and its ability to regulate precisely the intake of both protein and carbohydrate under a number of different situations is now well documented (reviewed in Simpson & Raubenheimer 2000). These include conditions where locusts are faced with different pairings of individually unbalanced but complementary foods (Chambers et al. 1995), different degrees of dilution of foods containing nutrients in near optimal ratios

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(Raubenheimer & Simpson 1993) and paired complementary foods occurring at different frequencies (Behmer et al. 2001). However, locusts are sometimes impeded from reaching their protein–carbohydrate intake target. For instance, when allelochemicals are present in the food, nutrient intake often represents a trade-off between ingesting the optimal protein–carbohydrate mixture and limiting allelochemical intake (Raubenheimer 1992; Simpson & Raubenheimer 2001; Behmer et al. 2002), and the same has been demonstrated for caterpillars (Slansky & Wheeler 1992). Work with the caterpillar *Manduca sexta* has also shown that protein–carbohydrate regulation can be affected by the presence of parasites (Thompson et al. 2001).

Our aim in the current study was to investigate how protein–carbohydrate regulation in locusts was affected by the distance between foods of differing nutrient content, and to discover whether the distance between such foods influenced a locust's foraging strategy with experience. We did this by placing individual fifth-instar nymphs into one of three differently sized circular arenas that contained four dishes of synthetic food (one dish contained a high-protein, low-carbohydrate food and the other three contained low-protein, high-carbohydrate food). Alone, these foods are suboptimal but together they are complementary, which means that locusts have to feed from both food types if they are to reach their intake target. Our small arenas were meant to emulate conditions of high plant density, where insects could quickly and easily move from one food dish to another. In the other two arenas, locusts had to move further to reach the different food dishes, a situation that emulated more patchy and open environments. For nymphs of the African desert locust, each of the simulated conditions is likely to be encountered under natural conditions. We measured food consumption at three intervals (days 0–2, 2–5 and 5–end of the stadium), which allowed us to quantify the development of nutrient intake and patterns of food consumption over time in each of the three arenas. Detailed behavioural observations were also made on each locust over the entire 12-h light phase on days 1 and 4. This permitted us to track movement patterns between the food dishes in the arenas and measure how foraging behaviour in the different-sized arenas changed with time or experience.

METHODS

Insects and Experimental Chambers

Locusts came from a culture kept under crowded conditions and maintained on a diet of seedling wheat and wheat germ at the Department of Zoology, University of Oxford, U.K.

The effect of distance between foods was tested in three circular Perspex arenas with diameters designated as small (20 cm), medium (40 cm) and large (80 cm). These arenas each stood 10 cm tall and were covered by a sheet of clear Perspex. The outsides of each arena were wrapped in white paper so that the locusts could not see each other.

The arenas stood on a paper floor marked with a grid to aid behavioural recordings.

Each arena contained four dishes of synthetic food (modified petri dishes specifically designed to minimize spillage; Raubenheimer & Simpson 1990) adjacent to the walls of the arenas and equidistant from each other. In the small arenas, the dishes that sat across from one another were 15 cm apart, and the dishes adjacent to one another were 10.6 cm apart, with measurements taken from the centre of the dishes. In the medium and large arenas, the distances between opposite dishes were 35 and 75 cm, respectively, and those between adjacent dishes were 25.8 and 53 cm, respectively. In each arena between the wall and each food dish was an expanded aluminium perch (approximately 5 × 2 cm) that provided a place for locusts to roost beside the food. Water for drinking was provided in a small plastic container (7 × 4 × 2 cm with two 1.5-cm holes in the top). The small arenas had a single, centrally positioned water container, which served all food dishes, and the medium and large arenas had a water container placed 6 cm from each food dish.

All experiments were conducted in a controlled temperature room at 29–31°C under a 12:12 h light:dark photoperiod. Because of limited bench space, we had to perform the experiment in two runs. For each experimental run, each arena size was replicated five times. Over the two runs, we used equal numbers of male and female locusts for each treatment ($N=5$ each).

Synthetic Foods

Dry, granular, chemically defined foods were made as described in Behmer et al. (2001). Two nutritionally complementary foods were used, defined according to the self-regulated diet of locust nymphs (Chambers et al. 1995). One food type (P-food) contained 31% protein (p) and 11% digestible carbohydrate (c), while the other (C-food) contained p7:c35.

Experimental Protocol

Each arena, regardless of size, had the same complement of food dishes: one dish of P-food and three dishes of C-food. Before the experiment, these foods had been allocated to their respective dishes and left to equilibrate to ambient room humidity levels (RH 30–40%) for about 24 h before being weighed (± 0.1 mg). Once the food dishes had been placed inside the arenas, freshly moulted fifth-instar locusts were weighed and released singly into arenas. They were then allowed to feed for 48 h, after which each dish was removed and replaced with a fresh, preweighed dish of the same food type. The food dishes that had been removed were allowed to equilibrate before they were reweighed. We repeated this protocol at the end of day 5 and day 8 and after the locusts had moulted to the adult stage (usually between days 9 and 11).

The wet mass of newly moulted adults was recorded and they were frozen. Carcasses were then dried in a desiccating oven at 40°C in preparation for determination

of total lipid and nitrogen content, using chloroform extraction and micro-Kjeldahl analysis (Behmer et al. 2001). To calculate the amount of lipid and nitrogen growth that occurred during the fifth instar, a separate group of newly moulted fifth-instar locusts was frozen and analysed to establish starting body lipid and nitrogen content. A regression equation was derived to estimate the starting body lipid and nitrogen content of the test insects.

Behavioural Observations

In addition to quantifying food consumption patterns, we monitored the behaviour of each locust for the duration of the 12-h light phase on days 1 and 4. We chose these days to evaluate the effects of time and experience on movements and patterns of foraging in the different-sized arenas. Observations began in the morning as soon as the lights came on (0800 hours). For each individual, we recorded, at 2-min intervals, its position in the arena, determined by which grid its head and thorax were in. If the locust was in a grid containing food, we recorded whether it was feeding, resting on the food but not feeding, or not on the food. From these observations, we were able to estimate overall activity levels, time budgets showing the time spent in specific grids, transitions between specific grids, the total distance moved by individuals within their arena, the length of feeding bouts at specific food dishes and meal durations.

The size of the food-containing grids was identical (about 64 cm²) in each arena, but the total nonfood-containing grids increased as arena size increased (64, 1000 and 4770 cm² for the small, medium and large arenas, respectively). This has two implications. First, the distance that a locust can locomote in a single direction will increase as arena size increases. Second, if the time that a locust spends in a given spot within an arena is determined by the simplest probability model (e.g. dividing the size of a grid by the total area of the arena), then the relative time that it is expected to spend in a food grid will decrease as arena size increases. It also means that, within a given arena size, locusts will spend equal amounts of time in each of the food-containing grids.

For all locusts, separate meals were identified when we observed feeding gaps of longer than 5 min (Simpson 1995). The duration of a meal was calculated by summing all feeding bouts and the pauses separating the feeding bouts within a meal. Locusts took most meals from a single food dish, but sometimes they took them from more than one food dish. When feeding bouts occurred at two dishes and the amount of nonfeeding time separating the two bouts was less than 5 min, we classified these as mixed meals (Simpson 1995). In addition to the types of meals being taken, we also recorded whether a specific feeding transition had occurred; the maximum number that any individual could make was 16.

Statistical Analysis

Measures of food consumption and insect performance were analysed using analysis of covariance (ANCOVA),

multivariate analysis of covariance (MANCOVA), repeated measures analysis (RMA) and failure time analysis (PROC LifeReg) techniques. For MANOVA analyses, we used Roy's Greatest Root, the MANOVA test statistic that has the greatest power (Scheiner 1993). All analyses were performed using SAS 6.12 (SAS 1990). When necessary, the data were log transformed to meet underlying assumptions. For most ANCOVA analyses, initial mass of locusts was used as a covariate to correct for size differences between locusts (e.g. on average, females are larger than males). Where significant effects were observed, post hoc comparisons were performed using contrasts. For MANOVAs, contrasts followed the techniques used by Scheiner (1993). Where multiple contrasts were made, alpha levels were adjusted using Bonferroni methods.

RESULTS

Food Consumption

Figure 1 shows the amounts of P- and C-food eaten over the entire fifth stadium (days 0–end) and for the three intervals (days 0–2, 2–5 and 5–end). Arena size significantly affected consumption only during the last period (days 5–end) (Table 1). Between day 5 and the end of the stadium, locusts from large arenas ingested more P- and C-food than locusts from medium or small arenas did (Fig. 1d). Linear contrasts revealed that, during this time, consumption of both P- and C-food increased linearly as the arena size increased. Although we found no significant difference in P- and C-food consumption between the arenas during the first two periods or over the entire stadium, we did observe a significant linear decreasing trend in P-food consumption, and a significant linear increasing trend in C-food consumption, respectively, as the arena size increased. Based on amounts eaten, the P-food dish in each arena was always the most preferred food dish, and the amount eaten from the P-food dish was usually equal to or greater than the sum of food eaten from the three C-food dishes. The total amount of food eaten was similar in all three arenas (ANCOVA: $F_{2,28}=0.70$, $P=0.507$), and we found no significant effect of sex ($F_{1,28}=1.16$, $P=0.293$) or sex-by-arena interaction ($F_{2,28}=1.33$, $P=0.286$).

Nutrient Intake, Growth and Performance

Figure 2a shows a bicoordinate plot of the amounts of protein and carbohydrate ingested for the three intervals (days 0–2, 2–5 and 5–end) and summed over the entire fifth stadium (days 0–end). The results exactly mirrored those previously obtained for consumption of the P- and C-food (Table 1). Locusts regulated their protein-carbohydrate intake to a statistically similar point except during the last period (days 5–end) (Table 1). Between day 5 and the end of the stadium, locusts from large arenas ingested more protein and carbohydrate than locusts from medium or small arenas. Linear contrasts revealed that, during this time, both protein and carbohydrate intake increased linearly as the arena size increased.

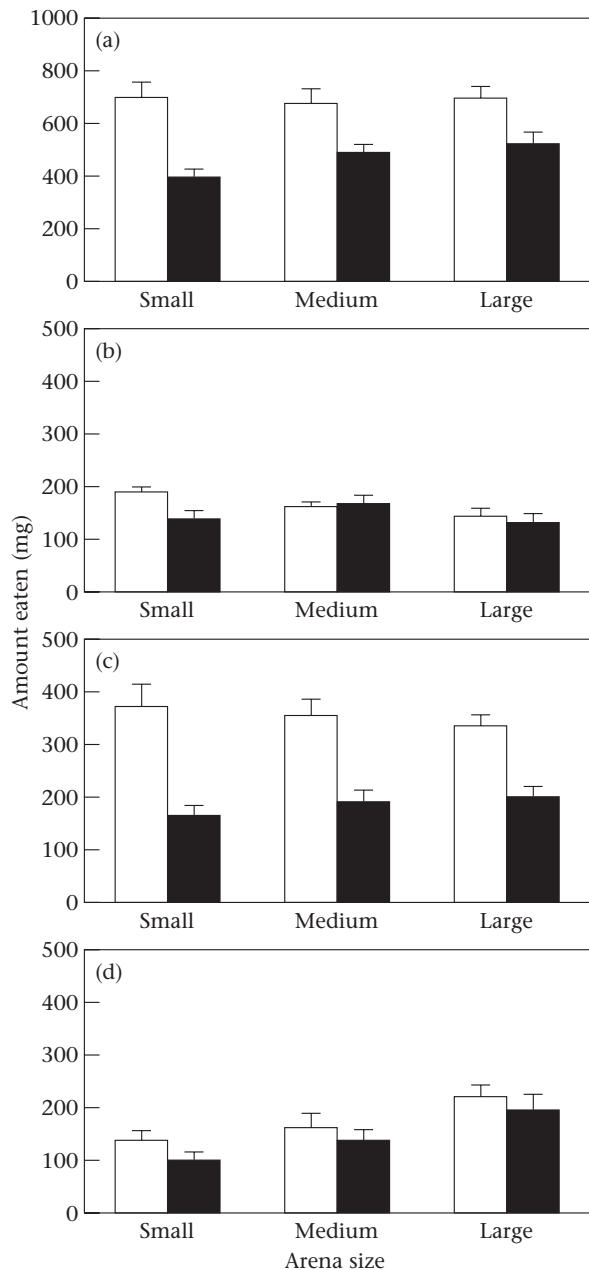


Figure 1. The mean \pm SE total amount of P-food (p31:c11; \square) and C-food (p7:c35; \blacksquare) eaten by locusts. (a) Consumption over the entire experiment (days 0–end); (b), (c) and (d): consumption over days 0–2, 2–5 and 5–end, respectively.

Although we found no significant differences in protein–carbohydrate intake across the arenas during the first two time periods, or over the entire stadium, we did observe a significant linear decrease in protein intake and a significant linear increase in carbohydrate intake as arena size increased.

Both developmental time (SAS Proc Lifereg: $\chi^2_2=4.32$, $P=0.115$) and mass gain (ANCOVA: $F_{2,28}=0.96$, $P=0.398$) were similar across the three treatments. Body lipid and nitrogen content also showed no significant differences across arena size (MANOVA: $F_{4,44}=1.87$, $P=0.133$; Fig. 2b). There was, however, a significant increasing linear

trend in body lipid content as arena size increased (linear contrast: $F_{1,44}=7.31$, $P=0.013$). No such trend was observed for body nitrogen content (linear contrast: $F_{1,44}=3.35$, $P=0.535$).

Feeding behaviour

Our detailed individual records of behaviour allowed us to document the effect of arena size on the manner in which locusts foraged between the available food dishes. For example, locust 7 (small arena, second run, first day) showed the following feeding sequence:

C1 \rightarrow C1 \rightarrow C1 \rightarrow C3 \rightarrow C1 \rightarrow (C1 + C2) \rightarrow C3 \rightarrow P \rightarrow P \rightarrow (C1 + P)

In this case, P refers to the dish containing the p31:c11 food, and C2 is the C-food dish opposite the P-food dish. The labelling for the two C-food dishes placed on either side of the P-food dish was determined by the number of feeds it received on the day that observations took place. Of these two dishes, the one that was fed from most frequently was called C1, and the other was called C3. Locust 7 was observed feeding from all available food dishes and in total took 10 meals, two of which were mixed meals (indicated by parentheses). For locust 7, we observed nine unique feeding transitions.

Number of food dishes eaten from. Locusts in the large arenas took feeds from fewer dishes than locusts from the small arenas (RMA: $F_{2,27}=4.10$, $P=0.028$). The number of dishes eaten from between days 1 and 4 decreased ($F_{1,27}=6.46$, $P=0.017$), and although there was no significant arena-by-day interaction ($F_{2,27}=1.49$, $P=0.243$), most of this time effect was because of a decrease in the number of food dishes eaten from in the medium and large arenas. In almost all cases, locusts ate from the P-food dish, so we focused on feeds from the C-food dishes. We found a significant difference in the number of C-food dishes eaten from in the different arena sizes (RMA, one-tailed: $F_{2,27}=3.00$, $P=0.034$) and a significant time effect ($F_{2,27}=5.84$, $P=0.023$), with most of the reductions again occurring in the medium and large arenas (Fig. 3).

Arena size. There were three possible effects on the types of meals being taken: feeding only from the P-food dish, feeding exclusively from a single C-food dish or taking a mixed meal (Fig. 4a, b). Day 1 showed a significant treatment effect (MANOVA: $F_{3,26}=8.71$, $P<0.001$), and univariate statistical analyses suggested that the primary difference between arenas was in the number of C-meals and mixed meals taken. The nature of these effects, as revealed by contrast analyses, was linear. For instance, as arena size increased, the number of C-meals taken increased ($F_{1,26}=5.52$, $P=0.027$). In contrast, the number of mixed meals taken decreased linearly as arena size increased ($F_{1,26}=7.25$, $P<0.012$). We also observed a significant treatment effect on day 4 (MANOVA: $F_{3,25}=6.70$, $P=0.002$), but here the main difference between the arenas was only in the number of mixed meals taken. Similar to the pattern seen on day 1, the

Table 1. Results of MANCOVAs for consumption of the P and C food and protein-carbohydrate intake

Source	df	F			
		Days 0-2	Days 2-5	Days 5-end	Entire stadium
P and C food consumption					
Arena	4,44	2.37	0.88	3.89**	1.93
Linear contrast (P-food)	1	7.01*	1.06	5.70*	0.05
Linear contrast (C-food)	1	2.00	1.32	9.92**	6.45*
Sex	2,21	1.63	0.70	0.37	0.46
Arena×Sex	4,44	0.68	1.35	0.56	0.71
Initial mass	2,21	1.85	3.26	8.46**	11.38**
Protein-carbohydrate intake					
Arena	4,44	2.35	0.91	3.85**	1.87
Linear contrast (Prt)	1	9.03**	0.62	10.10**	0.01
Linear contrast (CHO)	1	3.82	0.37	15.93**	4.60*
Sex	2,21	1.76	0.62	0.39	0.53
Arena×Sex	4,44	0.85	1.34	0.57	0.72
Initial mass	2,21	2.13	3.24	8.78**	10.83**

Overall *F* values (Pillai's Trace) are reported for the entire fifth stadium and each time interval. Initial mass was used as a covariate to adjust for size differences between insects. **P*<0.05; ***P*<0.01. Linear contrasts from one-way ANOVAs are reported for P- and C-food consumption and for protein (Prt) and carbohydrate (CHO) intake.

number of mixed meals taken decreased in a linear fashion as arena size increased ($F_{1,26}=20.43$, $P<0.001$). When we compared days 1 and 4 by measuring the change in the number of different meal types taken (positive values indicate an increase, negative values a decrease), we found a significant arena size effect (MANOVA: $F_{3,25}=3.23$, $P=0.039$; Fig. 4c); i.e. there was an increase and decrease in the number of P- and C-meals taken, respectively, in the large arenas, but no change in the number of P-, C- or mixed meals taken in the small arenas. Linear contrasts showed that, as arena size increased, the number of C- and mixed meals taken significantly decreased (two-tailed, $F_{1,26}=4.35$, $P=0.047$, and one-tailed, $F_{1,26}=4.97$, $P=0.018$, respectively), but there was a significant increase in the number of P-meals taken (one-tailed, $F_{1,26}=3.54$, $P=0.036$).

Types of feeding transitions. Figure 5 shows for each arena, on each day, the number of individual locusts that were observed making a particular feeding transition on at least one occasion. The most frequently observed feeding transition was the P-P transition, and on day 4 every locust made this transition at least once. By day 4, the number of different transitions that we observed occurring at least five times decreased as arena size increased (7, 4 and 3, in the small, medium and large arena, respectively). In the medium-sized arenas, most feeding transitions were concentrated on or between the P- and C1-dish, but in the large arenas, the most frequent observed transition, other than the successive P-food transition, was from the C1-dish to the P-dish, and from the C2-dish to the P-dish.

Feeding sequences. We counted the number of times that locusts took feeds from a food dish, then moved and fed from a different food dish. For instance, locust 7

had eight feed and move transitions, as indicated by the parentheses below (arrows between dishes indicate a new meal, pluses between dishes indicate a mixed meal):

(C1→C1→C1)→(C3)→(C1→C1)+
(C2)→(C3)→(P→P)→(C1)+(P)

When these data were analysed for each 12-h observation period, we saw a significant arena-by-time effect (RMA: $F_{2,27}=5.08$, $P=0.013$). On the first day of observations, locusts in the different arenas showed similar behaviour with respect to eating and then moving to a new dish (Fig. 6). On day 4, locusts in the large and medium arenas fed and moved at a similar rate as they had on day 1. In contrast, locusts in the small arenas fed and moved to a different food dish at a significantly higher frequency than locusts in the medium and small arenas.

Activity levels and time budgets

To gauge whether arena size influenced activity levels, we counted the number of times that locusts were observed locomoting. An individual was characterized as locomoting if it crossed from one grid to another. Although there was much variation, even between individuals from the same arena size, we found no effect of arena size (RMA: $F_{2,27}=0.73$, $P=0.494$) or day ($F_{1,27}=0.09$, $P=0.760$) on the activity levels of locusts from the different-sized arenas and no arena-by-day interaction ($F_{2,27}=0.68$, $P=0.515$). The total distance that locusts travelled over the course of the 12-h observation was calculated by summing the lengths of all its movements. There was a significant effect of arena size on total distance moved (RMA: $F_{2,27}=9.95$, $P<0.001$), but no effect of day ($F_{1,27}=1.02$, $P=0.321$) or arena-by-day interaction

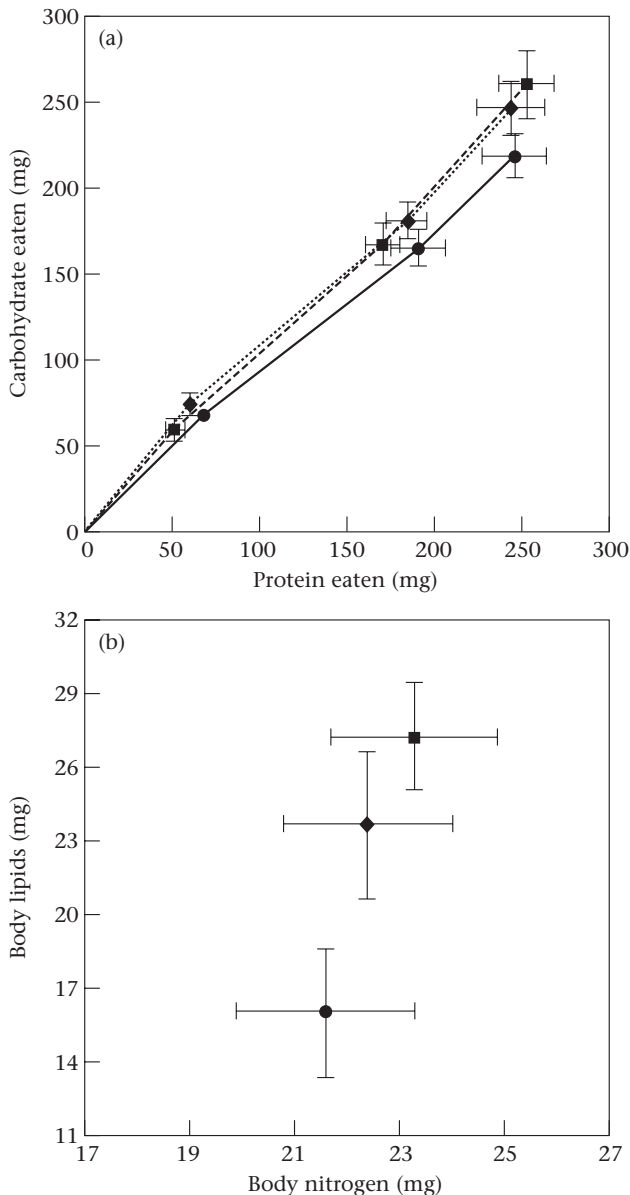


Figure 2. Bivariate means \pm SE for macronutrient intake and body growth. (a) The protein-carbohydrate intake trajectories for locusts from the small (—, ●), medium (---, ◆) and large (-.-, ■) arenas. The first, second and third points along each trajectory indicate the cumulative intake point reached after 2 days, 5 days and at the end of the stadium, respectively. (b) A bicoordinate plot of body nitrogen and lipid gain over the fifth stadium.

($F_{2,27}=0.95$, $P=0.400$). Distance travelled was greatest for locusts in the large arenas ($\bar{X} \pm \text{SD}=1826 \pm 262$ cm) and least for locusts in the small arenas (701 ± 95 cm).

Time budgets were constructed for individual locusts by summing the time that was spent in the different grids in the arenas (Fig. 7). On days 1 and 4, locusts from the small arenas had similar time budgets to what would have been expected based on random probability, and no significant change occurred in their time budgets over time (Fig. 7c). In contrast, the time budgets for locusts from the medium and large arenas did not follow a simple probability model, and locusts did not spend equal

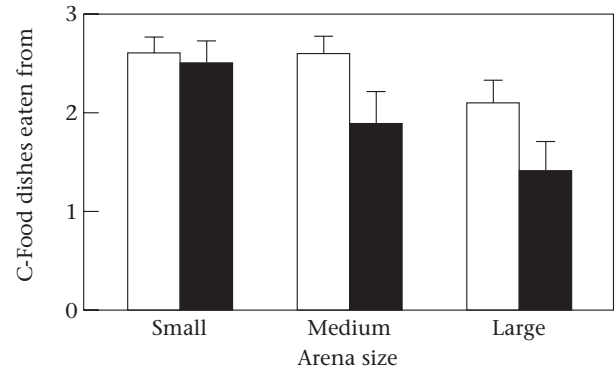


Figure 3. Mean \pm SE number of C-food dishes eaten from by an individual locust during the two observation periods: days 1 (□) and 4 (■).

amounts of time in the different food grids (Fig. 7). On day 1, for instance, locusts in the medium and small areas spent approximately 30% of their time in the P-containing food grid, and 45 and 30% of their time, respectively, in the C-containing food grids. Locusts from the medium and large arenas showed a significant change in time budgets between days 1 and 4 (MANOVA: $F_{3,26}=3.94$, $P=0.019$), most notably an increase in the time they spent in the P-containing food grid (Fig. 7c). For locusts from the medium arenas, this increase was paired with an equal decrease in the time spent in the C-food grids. In contrast, locusts from the large arenas equally decreased the time that they spent in the C-food and nonfood grids.

Although locusts in the medium and large arenas spent most of their time in the P-food grid, they still had to move to and feed from the C-food dishes to regulate their nutrient intake. We therefore examined whether arena size influenced locusts' time budgets with respect to how they distributed their time in the three C-food-containing grids. We created an index (I_C), that measured whether they distributed their time equally between the three C-food grids. The index was calculated as follows:

$$I_C = C_{\text{pref}} / (C_{\text{ttl}}) \quad (1)$$

where C_{pref} refers to the C-containing food grid where the most time was spent, and C_{ttl} refers to the combined amount of time spent in all three C-containing food grids. Values approximating 33% would indicate no preference for any of the C-containing food grids.

We found a significant interaction between arena size and time (one-tailed RMA: $F_{2,27}=3.20$, $P=0.028$). In the large arenas, the mean \pm SE I_C value was high on both day 1 ($80 \pm 4.5\%$) and day 4 ($72.1 \pm 4.9\%$), and in the medium arena, it increased from $62.2 \pm 3.8\%$ on day 1 to $75.2 \pm 7.4\%$, on day 4. The I_C value was lowest in the small arenas and dropped from $55.4 \pm 4.1\%$ to $48.6 \pm 1.8\%$ between days 1 and 4.

DISCUSSION

The foraging success of an animal can be measured in a number of ways. These include the method that it uses to

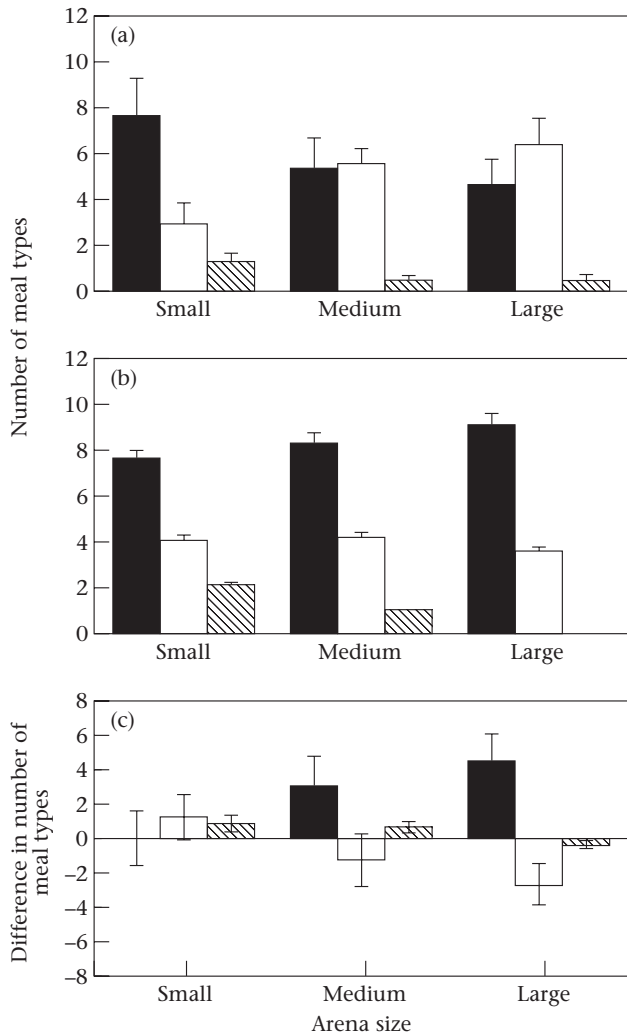


Figure 4. The mean \pm SE number of different meal types taken by locusts in the different arenas on (a) day 1, (b) day 4. (c) The difference between days 1 and 4. A meal could be made up of a single feeding bout or multiple feeding bouts as long as the amount of nonfeeding separating the feeding bouts was not greater than 5 min. Three types of meals existed: P-food meals (p31:c11; ■), C-food meals (p7:c35; □; this occurred exclusively from a single C-food dish) and mixed meals (meals that consisted of feeding bouts from more than one dish; ▨).

search in relation to the availability of resources and their distribution in the environment, its efficiency in locating resources and its ability to respond through experience to its environment (Bell 1991). For herbivores, an additional measure of success is how well they regulate the intake of important nutrients. We found that the distance separating available foods did not significantly affect protein-carbohydrate intake in locusts, and that the intake points attained by locusts in the three arenas closely matched their preferred 'intake target', which studies have shown to approximate p21:c21 (Chambers et al. 1995; Behmer et al. 2001). As a result, locusts in the three different-sized arenas grew and developed at similar rates. However, both consumption and behavioural data revealed that locusts adopted different foraging strategies depending

on the distance separating the foods. There was also a suggestion that, in the larger arenas, learning might have been important in modifying foraging strategies.

That locusts in the three sizes of arena tightly regulated their protein-carbohydrate intake and reached the intake target adds to the growing evidence that locusts can show this behaviour under a number of scenarios. This evidence includes responses to nutrient dilution (Raubenheimer & Simpson 1993), paired nutritionally unbalanced but complementary food items (Chambers et al. 1995) and changing food frequency (Behmer et al. 2001). Such regulatory ability is probably the result of an interaction between their sensory capabilities and their internal physiological state, and can involve learning (reviewed in Simpson & Raubenheimer 2000).

Results suggest, however, that the distance between foods has a subtle effect on nutrient regulation, since we observed significant linear trends in the amount of protein eaten during the first (days 0–2) and last (days 5–end) feeding periods, and increasing trends in the amounts of carbohydrate eaten during the last feeding period and over the entire fifth-stadium. Ultimately, the trends related to protein intake were balanced over the entire stadium, but we considered the possibility that the trend of increasing carbohydrate intake as arena size increased had a functional basis, reflecting a need by locusts to expend more energy while foraging in the larger arenas. However, two analyses fail to support this hypothesis. First, we found no positive relation between either activity levels or distance moved and the amount of carbohydrate consumed. Second, body lipid levels increased linearly as arena size increased. Had energy demands been greater in the large arenas, ingested carbohydrates would have been respired. The implication is that locusts respond to greater spacing between foods by increasing storage of lipid, which is a possible example of a feedforward regulatory response (Toates 1980).

Although distance did not have a long-term effect on nutrient regulation, the high frequency of mixed meals taken in the smaller arenas, compared with the medium and large arenas, suggests that distance might influence nutrient regulation in the short term. Similar patterns in the rate of switching as the distance between food increases have been found in other studies with grasshoppers (Bernays et al. 1997; van der Zee et al. 2002). This behaviour may reflect the fact that moving between foods, over an open area, constitutes a predation risk. There were no predators in our experiment, but predator avoidance may be an inherent behavioural trait, particularly for species that rely on crypsis as an antipredator defence. On the other hand, field studies suggest that chemically defended species may be less affected by the distance separating foods (Bernays & Raubenheimer 1991; Raubenheimer & Bernays 1993; Chambers et al. 1996).

Although locusts from the different arenas arrived at similar protein-carbohydrate intake points, they could have done so without having to adopt a specific movement strategy. That is, they might have been moving in an undirected manner with respect to the position and nature of the foods while making appropriate feeding

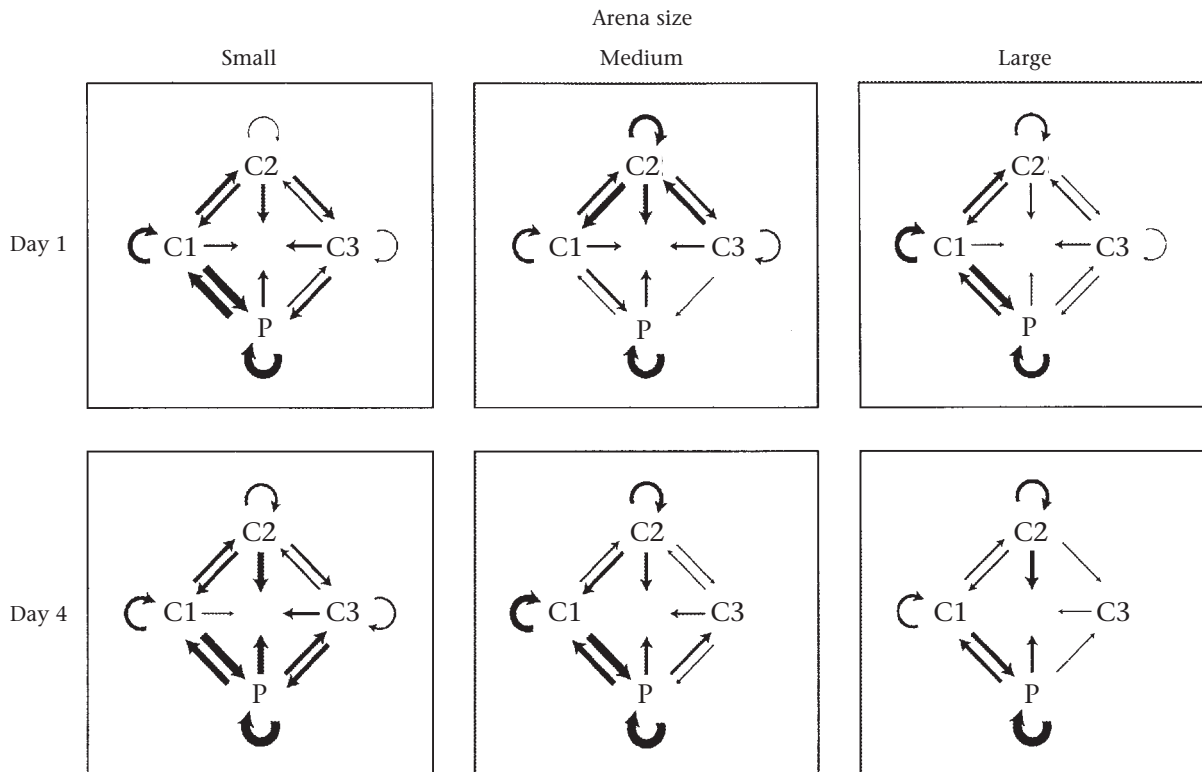


Figure 5. Summary of feeding transitions between the four dishes of food in the three arenas, on days 1 and 4. Each arena had one dish of P-food (p31:c11) and three dishes of C-food (p7:c35). The two C-food dishes adjacent to the P-food dish were coded either C1 (the more preferred of the two C-food dishes adjacent to the P-food dish) or C3 (the less preferred). C2: the C-food dish opposite the P-food dish. Thickness of arrows indicates how many locusts ($N=10$ per arena per day) made a particular transition at least once over the 12-h observation period. For reference, all locusts from the small, medium and large arenas on day 4 had a meal on the P-food dish, then returned to the P-food dish for their next meal.

decisions after reaching the foods (Simpson et al. 1991; Simpson & Simpson 1992; Dukas & Bernays 2000). Such 'random' movement strategies are often inferred from studies that follow herbivorous insects in the field (Cain 1985; Cain et al. 1985; Raubenheimer & Bernays 1993). However, most animals, including locusts (Simpson & White 1990; Dukas & Bernays 2000), use some degree of orientation towards foods over a distance in their searching behaviour (Morris & Kareiva 1991). None the less,

Morris & Kareiva (1991) advocated that the random element of foraging should not be overlooked, since it may work in concert with nonrandom search mechanisms to enhance the effectiveness of the search process.

To explore how the distance between foods influenced the extent to which foraging movements were directed, we compared locust settlement patterns in the different arenas. Since locusts in our study showed antiapostatic food selection (Greenwood 1984; Pennings et al. 1993; Behmer et al. 2001), they might be expected to tend to settle in the area of the arena containing the p31:c11 food and use this as a reference point for directing forays to other food dishes. Initially there was little difference between the three treatments in the time spent in the p31:c11 food grid, possibly because locusts entered the arenas naïve to the types of foods present and their distribution. By day 4, however, locusts in the medium and large arenas spent about 50% of their time in the grid containing the p31:c11 food and about 20% of their time in the three grids containing the p7:c35 foods. In contrast, locusts in the small arenas spent about 30% of their time in the p31:c11 food grid and about 65% of their time in the p7:c35 grids. For locusts in the small arenas, these values were nearly identical to those observed on day 1 and were similar to what would be expected if locusts settled in the food grids based on random probability.

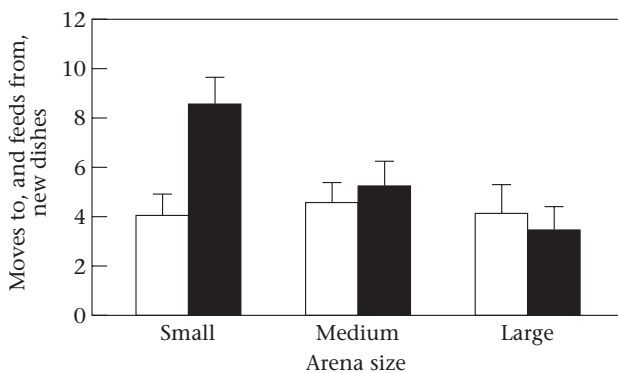


Figure 6. The mean \pm SE number of times locusts fed and then moved to a new food dish over a 12-h observation period on days 1 (□) and 4 (■).

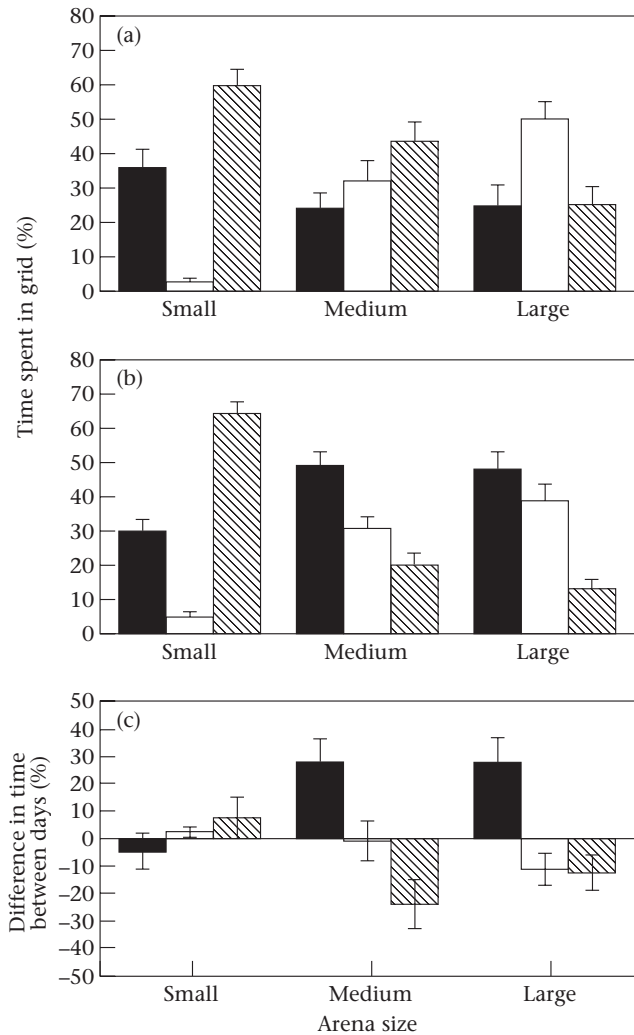


Figure 7. The mean \pm SE time spent by locusts in different grids within the arenas. Each arena had one P-food (p31:c11; ■) grid and three C-food (p7:c35; ▨) grids, and the size of each food grid was identical. The number and total size of the nonfood-containing grids (□) increased as the size of the arena increased. (a), (b) The amount of time, expressed as a percentage of the total observation time (12 h) spent in each of the three grids. (c) The change between days 1 and 4 in the time spent in a particular grid. A positive number indicates more time spent in a grid type on day 4, and a negative number indicates a decrease in this time.

There are two possible explanations for these results. First, learning might have been involved in the modification of patterns of movement between foods in the medium and larger arenas, but was hindered by the close proximity of foods in the small arenas. Second, some more passive mechanism may explain the pattern, with locusts more likely to rest near the P-dish after taking a meal, because of the greater inhibitory influence of ingested protein than carbohydrate on postprandial locomotion (Abisgold & Simpson 1987). Learning, especially associative learning, is well documented in herbivores (Gillingham & Bunnell 1989; Simpson & White 1990; Bernays 1995; Edwards et al. 1998a, b; Behmer & Elias 1999; Behmer et al. 1999; Dukas & Bernays 2000), but it

often requires reinforcement. In the medium and large arenas, this reinforcement would have existed since locusts often rested on their foods, or the perches behind the foods, after they had finished a meal. In contrast, the perches in the small arenas overlapped one another, much in the same way that plants do when they occur at high density. We regularly observed locusts walking along the top of the perch after they had fed. With no definable physical boundary separating the foods, and having walked along the top of the perch, the food dish that was encountered after a rest on the perch was often different from the one previously fed upon. This would have made it difficult for the locusts to associate nutritional consequences of eating a food with its position in the arena.

Even though locusts in the larger arenas tended to spend significantly more time in the grid containing the p31:c11 food, they still needed to leave that grid and feed from at least one of the p7:c35 food dishes if they were to balance their nutrient intake. If our locusts were perfectly efficient foragers, they would have regulated their nutrient intake by partitioning feeding between the p31:c11 food dish and one of the nearest p7:c35 food dishes. Although complete fidelity to a single C-dish did not develop in any of the treatments, locusts in the medium and large arenas visited fewer of the C-food dishes and made fewer unique transitions between food dishes between days 1 and 4. Perhaps with experience, locusts in the medium and large arenas were able to form a spatial representation of the different foods in the arenas and adjust their foraging behaviour based on this information. Such foraging strategies have been observed in mammalian herbivores (e.g. Gillingham & Bunnell 1989; Edwards et al. 1998b), and evidence suggests that insect herbivores may have similar abilities (Gallistel 1994; Collett & Zeil 1998).

Our results have shown that locusts modify their foraging behaviour to compensate for the spatial distribution of food sources, and in so doing maintain a consistent nutritional outcome. Of course, our manipulations were small-scale, compared even with the distances over which locusts forage (Bernays & Raubenheimer 1991; Raubenheimer & Bernays 1993; Chambers et al. 1996). None the less, the fact that even at these small scales the insects demonstrated distinct differences in foraging behaviour suggests that the scale of our experiments was meaningful to locusts. Were we to increase appreciably the spacing of our foods, we would at some point reach distances that exceed the compensatory ability of our animals and force them into decisions of habitat choice. This logic heads to the questions of how much nutritional perturbation an animal would tolerate before choosing between habitats, and how this would scale with such factors as the size of the animal and its host range. Such questions probe the exciting interface between regulatory physiology and animal ecology.

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