A comparison of nutritional regulation in solitarious- and gregarious-phase nymphs of the desert locust *Schistocerca gregaria*

S. J. Simpson*, D. Raubenheimer, S. T. Behmer, A. Whitworth and G. A. Wright

Department of Zoology and University Museum of Natural History, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

*e-mail: stephen.simpson@zoo.ox.ac.uk

Summary

Nutritional regulatory responses were compared for the cryptic 'solitarious' and the conspicuously coloured, aggregating 'gregarious' phases of the desert locust *Schistocerca gregaria*. The desert locust has the genetic potential to exist in either phase, changing between them within a lifetime and epigenetically across generations. Our aim was to compare final-instar nymphs of the two phases with respect to key nutritional variables, including (i) points of regulated intake (the 'intake target') for protein and carbohydrate, (ii) the nature of trade-offs between over-eating nutrients in excess and under-eating those in deficit when fed nutritionally unbalanced foods, (iii) diet-related patterns of nutrient utilisation, and (iv) the performance consequences of eating nutritionally unbalanced diets. When provided with pairs of nutritionally unbalanced but complementary foods, both phases regulated their intake of protein and carbohydrate to a similar point. However, when confined to foods that were of unbalanced protein to carbohydrate ratio, gregarious nymphs ate more than solitarious insects. Both phases regulated protein growth, but gregarious insects did so to a lower adult body protein content and converted ingested protein to growth less efficiently. When fed a food high in carbohydrate and low in protein, gregarious nymphs deposited more body lipid and survived less well than did solitarious insects. Solitarious nymphs developed more quickly than gregarious nymphs except on the two most extremely unbalanced diets, on which development time was similar. The results are discussed with respect to the different nutritional ecologies of the two phases and used to develop the hypothesis that animals have evolved to trade-off the cost of eating excess of a nutritionally unbalanced diet against the probability of encountering foods of complementary composition in the future.

Key words: *Schistocerca gregaria*, desert locust, phase, maternal inheritance, nutrition, feeding, behaviour.

Introduction

The desert locust *Schistocerca gregaria* has the genetic potential to exist in either of two extreme forms, phases *solitaria* or *gregaria*, which differ markedly in behaviour, colour, shape, metabolic physiology, life-history and ecology (Uvarov, 1966, 1977; Pener, 1991; Pener and Yerushalmi, 1998; Simpson et al., 1999). Solitarious *S. gregaria* are cryptically coloured, relatively inactive and are repelled by other locusts. They live at low population densities in desert environments that are typically dominated by a small number of host-plant species (Ghaout et al., 1991). Gregarious-phase insects, in contrast, are adapted to live at high population densities. As nymphs, they are conspicuously coloured, highly mobile and actively aggregate, producing marching bands (Kennedy, 1939; Ellis, 1951; Roffey and Popov, 1968). Phase change occurs both within the life of an individual in response to local population density and also accrues across generations *via* epigenetic parental effects (Pener, 1991; Pener and Yerushalmi, 1998; McCaffery et al., 1998). Such responses are consistent with models of the evolution of phenotypic plasticity and maternal inheritance in environments that remain in one state for prolonged periods but change occasionally and unpredictably into an alternative state, which may be sustained for several generations before reverting to the default condition (Jablonka and Lamb, 1998; Mousseau and Fox, 1998).

Sub-Saharan Africa provides exactly such an environment, being dry desert for long periods but experiencing flushes of vegetation following unpredictable and rare bouts of prolonged rains. When conditions are poor and population densities low, the best strategy is for locusts to remain cryptic. As conditions improve following rain, population size grows yet insects may remain camouflaged and isolated among the abundant vegetation. However, as conditions once more deteriorate, locusts become concentrated into condensing islands of vegetation and are forced together despite their predisposition to avoid each other (Kennedy, 1939; Roffey and Popov, 1968).

Physical contact between locusts provides the major stimulus for triggering phase-transition, inducing rapid behavioural changes that include increased levels of activity

and active aggregation (Roessingh et al., 1998; Hägele and Simpson, 2000; Simpson et al., 2001). Other changes in colour and morphometry develop over several stadia and accumulate across generations (Pener, 1991). Individual-based simulations (Collett et al., 1998) and laboratory and field experiments (Bouaïchi et al., 1996; Despland and Simpson, 2000a,b; Despland et al., 2000) have shown that the small-scale spatial distribution, abundance and nutritional quality of vegetation interact in determining the extent to which a local population of solitarious locusts gregarises. Local aggregations of conspicuously coloured gregarious nymphs may be protected from predation by feeding on toxic vegetation until such aggregations coalesce into swarms large enough to swamp predator numbers (Sword, 1999; Sword et al., 2000).

Given the striking differences in their behaviour, physiology and ecology, it might be expected that the two phases of *S. gregaria* would differ in their feeding and nutritional responses. Feeding behaviour serves the need of an animal for multiple nutrients. In our previous work, we have found that the two major nutrient groups that are regulated behaviourally and post-ingestively in locusts are protein and carbohydrate (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 2000). Our aim in the present study was to compare gregarious- and solitarious-phase locust nymphs with respect to three fundamental nutritional variables: (i) their intake requirements for protein and carbohydrate, (ii) the nature of trade-offs between over-eating nutrients in excess and under-eating those in deficit when faced with nutritionally unbalanced foods, and (iii) the efficiency with which ingested nutrients are utilised for growth.

Locusts were subjected to one of seven dietary regimes, and their food consumption, performance and nutrient utilisation were measured across the final nymphal stadium. In two of these treatments, insects had a choice between two nutritionally complementary foods, while in the other five treatments they were restricted to a single food. The experiments were conceived and the data interpreted using our Geometric Framework (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993). The choice treatments were used to establish the point to which nymphs would regulate their protein and carbohydrate intake when free to do so. The position of this 'intake target' (*sensu* Raubenheimer and Simpson, 1993) was compared between the phases and used as the reference for interpreting patterns of consumption in nymphs restricted to single foods. Bi-coordinate plots of intake and growth (utilisation plots) (see Raubenheimer and Simpson, 1994) were then derived and compared to assess the efficiency with which ingested protein and carbohydrate were converted to growth.

Materials and methods
Insects
Experimental locusts *Schistocerca gregaria* (Forskål) were either crowd-reared for many generations ('gregarious phase') or from the same stock but solitary-reared for three generations ('solitarious phase'), according to the methods described by

Roessingh et al. (1993). In an additional experiment, one-generation solitary-reared nymphs were used (i.e. isolated since hatching from eggs laid by crowd-reared parents). Both the crowded and solitary cultures were fed a diet of greenhouse-grown seedling wheat and wheat germ. Nymphs of both sexes were collected from the cultures at ecdysis to the final nymphal stadium (day 0). Solitary-reared nymphs exhibited the green colour and characteristic morphometry of phase *solitaria*, while crowd-reared nymphs were conspicuously colour-patterned (black, yellow, white and orange), with a morphometry typical of phase *gregaria*. For photographs of animals from the Oxford cultures, see Simpson et al. (1999). 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 (modified 5.5 cm Petri dishes filled with approximately 2.0 g of synthetic food). A tissue culture flask perforated with a 1.5 cm diameter hole provided a water source. Insects were kept at 29–31 °C under a 12 h:12 h light:dark regime. Being placed alone into an arena results in partial behavioural solitarisation of initially gregarious locust nymphs. This change occurs within the first 4 h of isolation, and thereafter the insects remain in a stable, significantly gregarised behavioural state for the rest of the stadium (Roessingh and Simpson, 1994).

Diets
Locusts were allocated to one of seven diet treatments. Five 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 and 35:7. The others were two-choice treatments, one with 28:14 *versus* 14:28 and the other 14:7 *versus*. 14:28. These diet compositions were chosen on the basis of extensive previous work on locusts in our laboratory. 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 mixture of sucrose and white dextrin, while the protein contained 3:1:1 casein:peptone:albumen.

Protocol
In the main experiment comparing gregarious with three-generation solitarious nymphs, representatives of all treatments were run concurrently, with the experiment being replicated five times to yield a total of 12–20 locusts per treatment. The following variables were recorded for individual insects. (i) Initial wet mass, from which initial dry mass, lipid and protein content were estimated from regression equations established from a sample of 20 insects (10 solitarious and 10 gregarious) killed immediately after ecdysis to the fifth stadium. (ii) Dry mass of food consumed over 3-day periods from day 0 until adult ecdysis or death. Consumption was derived from the mass change in the food dishes. The dry mass of food eaten, in turn, provided a measure of the amount of protein and carbohydrate consumed. (iii) Stadium duration or time to death during the fifth stadium, measured to the nearest day. (iv) Dry mass, lipid and

nitrogen content of those insects surviving to adulthood. Newly ecdysed adults were killed by freezing and dried to constant mass in a desiccating oven at 40 °C. Carcasses were weighed to the nearest 0.1 mg, lipid-extracted in three 24 h changes of chloroform, re-dried and re-weighed, then analysed for nitrogen content using the micro-Kjeldahl procedure. (v) Protein and lipid growth, estimated for each insect from initial wet mass on day 0 and adult composition. When converting from nitrogen to protein content, a conversion factor of 6.25 was used.

An additional experiment was run in which one-generation solitary-reared locusts were provided with the same no-choice diet treatments as above. Intake was measured across the final nymphal stadium for 10 insects (five male and five female) per treatment.

Statistical analyses

Aside from percentage survivorship, only data from insects that survived to adulthood were analysed. Data analysis was undertaken using the General Linear Model (GLM) facility in SPSS (version 9.0) and SAS (6.12).

Results

Position of the intake target

When provided with a choice of foods, locusts regulated their intake of both protein and carbohydrate, with neither protein nor carbohydrate intake differing between the two treatments [$F_{1,34}$ (*P*)=1.24 (0.274), 1.61 (0.213), 1.52 (0.226) and 2.82 (0.102) for protein intake across days 0–3, 0–5, 0–8 and across the entire stadium, respectively; and 0.68 (0.414), 1.44 (0.239), 2.36 (0.134) and 1.73 (0.197) for carbohydrate intake over the same periods, with initial mass of the locust at the beginning of the stadium included as a covariate]. Accordingly, data were pooled across the two choice treatments for each phase of locust, and the resulting mean was used as the estimate of the intake target (regulated point of protein and carbohydrate intake). Neither protein nor carbohydrate intake differed between the two phases of locust, either as a main effect [$F_{1,34}$ (*P*)=0.77 (0.387), 2.69 (0.110), 1.04 (0.316) and 0.26 (0.616) for protein intake across days 0–3, 0–5, 0–8 and across the entire stadium, respectively; and 0.53 (0.474), 2.01 (0.166), 0.33 (0.570) and 0.12 (0.728) for carbohydrate intake] or as an interaction with food treatment [$F_{1,24}$ (*P*)=0.08 (0.783), 0.04 (0.852), 0.01 (0.929) and 0.20 (0.661) for protein intake across days 0–3, 0–5, 0–8 and across the entire stadium, respectively; and 0.04 (0.850), 0.092 (0.763), 0.03 (0.875) and 0.09 (0.772) for carbohydrate intake].

Nutrient balancing

Whereas data from the choice experiments indicated no significant difference in the protein–carbohydrate intake targets for the two phases, when forced to eat a single food containing a protein to carbohydrate ratio that differed from the self-selected ratio, the two phases showed markedly different intakes when balancing under-eating one nutrient
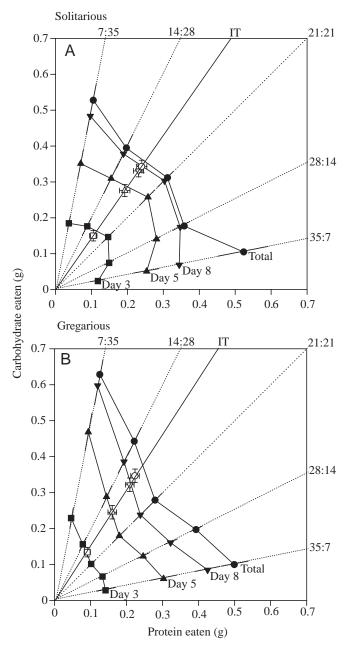

Fig. 1. Protein–carbohydrate intake arrays for solitarious (A) and gregarious (B) desert locust nymphs. Filled symbols represent intake points for locusts provided with a single food, whose protein and digestible carbohydrate content is indicated at the end of each dotted line (%protein:%carbohydrate). Open symbols show the mean intake points for locusts provided with a choice of either food 28:14 with food 14:28, or food 14:7 with food 14:28. Values are means ± S.E.M. (*N*=7–20). Intake did not differ between these latter two treatments, indicating regulation of protein–carbohydrate intake. This selected point of intake ('intake target', labelled IT) provides a reference for interpreting the intake array across the no-choice treatments (see Fig. 2). The data indicate cumulative intake from ecdysis (day 0) until day 3 (squares), day 5 (triangles), day 8 (inverted triangles), and across the entire stadium until adult ecdysis (circles).

group and over-eating the other (Fig. 1). The mass of food eaten (and hence points of protein–carbohydrate intake, given

that all diet treatments contained 42 % macronutrients) differed significantly between the two phases when insects were constrained from reaching the intake target [phase × food treatment interaction term in GLM with food eaten as a dependent variable: $F_{4,114}$ (*P*)=2.45 (0.05), 4.06 (0.004) and 3.27 (0.014), for days 0–3, 0–5 and 0–8, respectively, with initial mass of the locust at the beginning of the stadium included as a covariate]. Across the entire stadium, protein and carbohydrate intake differed between the phases, but there was no significant interaction with dietary treatment [$F_{1,114}$ (*P*)=17.78 (<0.0001) for the main effect of phase, and $F_{4,114}$ (*P*)=1.56 (0.190) for the interaction with diet].

As can be seen from Fig. 1A, the intake array for solitarious locusts across the no-choice treatments was arc-shaped up until day 8 because the insects ingested less food as the protein–carbohydrate ratio became more imbalanced. By day 8, the locusts on the more balanced foods had ceased eating in advance of ecdysis to adulthood. Nymphs on the two extreme foods (7:35 and 35:7) had not ingested sufficient nutrients to moult, however, and continued to eat beyond day 8, thus prolonging their development time and causing the intake array to straighten. Such a pattern of intake indicates that solitarious locusts followed a strategy of minimising the absolute sum of over-eating the nutrient in excess and under-eating the deficient nutrient relative to the intake target; a balancing rule we have previously called the 'closest distance rule' to indicate the fact the animals are moving to the closest point to the intake target in the available nutrient space (Raubenheimer and Simpson, 1997) (Fig. 2).

In contrast, the intake array for gregarious locusts was not arc-shaped at any stage of development, but instead was more linear throughout the stadium (Fig. 1B). Gregarious insects ingested more of the most unbalanced diets than did solitarious locusts (Fig. 2). Hence, whereas by the end of the stadium gregarious and solitarious insects had attained somewhat similar protein and carbohydrate intakes (Fig. 1), they achieved this by very different time courses of intake throughout the stadium.

Nutrient balancing in one-generation solitary-reared nymphs

To explore the transition between the solitarious (three-generation solitary-reared) and gregarious responses, an extra no-choice experiment was run with one-generation solitary-reared nymphs. These animals showed a pattern of intake that was transitional between arrays for three-generation-solitary and crowd-reared nymphs (Fig. 3). Their intake array was asymmetrical because the insects ate a small amount of 7:35 diet relative to 35:7.

Nutrient utilisation

Patterns of nutrient utilisation across the stadium differed between the two phases (Fig. 4; note that intake data are across the entire stadium, hence are based on points labelled 'total' in Fig. 1). Analysis of covariance with protein growth as the dependent variable and protein intake as the covariate indicated that there were significant covariate by factor interactions (i.e.
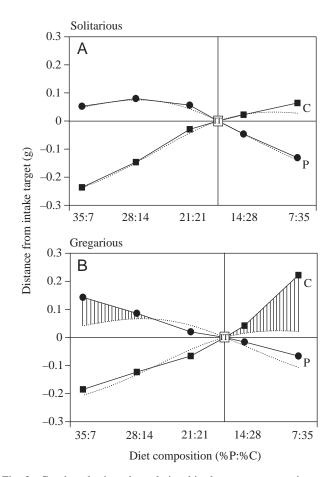

Fig. 2. Graphs plotting the relationship between over-eating one nutrient (protein, P, or carbohydrate, C) and under-eating the other *versus* food composition, relative to the selected point of intake when insects were provided with a choice of complementary foods (the intake target, IT, from Fig. 1). Data are for intake up to day 5 in the stadium. The theoretical minimum nutritional error (absolute sum of over-eating one nutrient and under-eating the other relative to the intake target) is shown as a dotted line. Note how solitarious insects (A) followed a strategy that minimised the total nutritional error, while gregarious locusts (B) tolerated over-eating the nutrient in excess relative to the intake target (hatched region).

slope differences) for both phase and dietary treatment [$F_{1,94}$ (*P*)=17.2 (<0.0001) and $F_{4,94}$ (*P*)=3.01 (0.022), respectively]. The same was true for lipid growth *versus* carbohydrate intake [$F_{1,94}$ (*P*)=12.4 (0.001) and $F_{4,94}$ (*P*)=8.62 (<0.0001), respectively]. There was, however, no significant three-way interaction between intake, phase and diet [$F_{4,94}$ (*P*)=1.15 (0.339) and 0.70 (0.596) for protein and lipid utilisation, respectively].

It is apparent from Fig. 4 that both phases regulated their protein growth, with the level of body protein stabilising at intakes in excess of approximately 0.2 g of protein. Insects on the choice treatments (the triangles in Fig. 4) ingested close to the minimum amount of protein (approximately 0.2 g) needed to achieve the regulated level of protein growth. Insects that were forced to ingest an excess of protein (>0.2 g) by being confined to diets with a high protein to carbohydrate ratio did
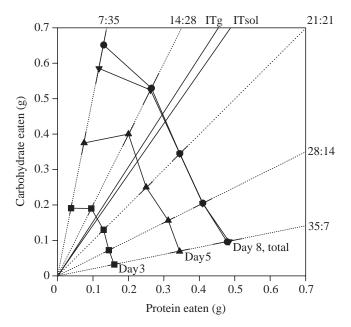
Fig. 3. Protein–carbohydrate intake array for one-generation solitary-reared desert locust nymphs. Refer to Fig. 1 for explanation. Values are means ± S.E.M. (*N*=9–10). ITg and ITsol indicate the self-selected protein–carbohydrate ratios for gregarious and three-generation solitarious nymphs, respectively (from Fig. 1). Data indicate cumulative intake from ecdysis (day 0) until day 3 (squares), day 5 (triangles), day 8 (inverted triangles), and across the entire stadium until adult ecdysis (circles).

not convert the excess to growth, presumably losing it through excretion.

However, gregarious locusts regulated to a lower adult body protein level than did solitarious insects. When the 7:35 diet was excluded from the analysis, protein growth was higher in solitarious than in gregarious locusts (0.123±0.004 g for gregarious *versus* 0.150±0.007 g for solitarious insects; means ± S.E.M., *N*=54 for solitarious and *N*=42 for gregarious insects), but did not differ with diet, either as a main effect or as an interaction with phase [$F_{1,88}$ (*P*)=7.545 (0.007) for the effect of phase; $F_{3,88}$ (*P*)=0.937 (0.426) for diet treatment, and $F_{3,88}$ (*P*)=0.516 (0.672) for the diet by phase interaction]. The difference in body protein content corresponded with gregarious insects weighing less as newly moulted adults [0.299±0.008 mg dry mass for gregarious *versus* 0.349±0.011 mg for solitarious insects; means ± S.E.M., *N*=69 for solitarious and *N*=56 for gregarious insects; $F_{1,123}$ (*P*)=13.41 (<0.001)], which in turn was explained by their smaller initial mass at the beginning of the stadium [0.567±0.01 g wet mass for gregarious *versus* 0.639±0.0176 g for solitarious nymphs; means ± S.E.M., *N*=69 for solitarious and *N*=56 for gregarious insects; $F_{1,123}$ (*P*)=11.15 (0.001)]. When initial mass was include as a covariate in the analysis, the statistical difference in final mass between the phases disappeared [$F_{1,122}$ (*P*)=2.59 (0.11) for the effect of phase, with $F_{1,122}$ (*P*)=188.8 (<0.0001) for initial mass as a covariate].

Despite gregarious insects being smaller as adults and regulating to a lower adult body protein content, they ingested
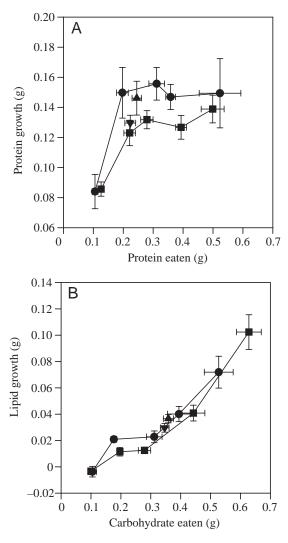

Fig. 4. Utilisation plots for protein growth across the entire fifth stadium *versus* total protein intake (A) and for lipid growth *versus* total digestible carbohydrate intake (B). Circles are for solitarious locusts and squares are for gregarious insects. Values are means ± S.E.M. (*N*=7–20). The points from left to right along each curve in the protein utilisation plot are for foods 7:35, 14:28, 21:21, 28:14 and 35:7; for the lipid utilisation plot, the order is 35:7, 28:14, 21:21, 14:28 and 7:35 (see Materials and methods). Triangular symbols indicate the points achieved by insects provided with a choice of complementary foods and, thus, able to reach their intake target. Triangles are for solitarious insects and inverted triangles for gregarious locusts.

a similar amount of protein across the stadium to that eaten by solitarious insects when intake across all diet treatments was pooled [0.330±0.019 g for gregarious *versus* 0.306±0.021 g for solitarious nymphs; means ± S.E.M., *N*=69 for solitarious and *N*=56 for gregarious insects; $F_{1,123}$ (*P*)=0.618 (0.433), not including initial mass as a covariate]. Hence, gregarious locusts utilised ingested protein for growth with lower efficiency.

In contrast to protein, body lipid levels were not strongly regulated (Fig. 4). There was no lipid growth on diet 35:7, whereas body lipid increased at a rate of approximately 0.25 g
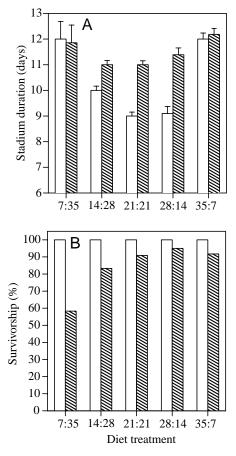
Fig. 5. Graphs showing development time (A) and percentage survivorship (B) across the fifth stadium for solitarious (open columns) and gregarious (hatched columns) locusts confined to foods containing a fixed proportion of protein (P) and digestible carbohydrate (C) (diet treatment labelled as %P:%C). Values are means + S.E.M. (N=7–20 in A, 12–20 in B).

per gram of carbohydrate eaten when total intake of carbohydrate exceeded approximately 0.3 g. Solitarious insects had a marginally higher lipid content than did gregarious insects across carbohydrate intakes from approximately 0.2 to 0.45 g [when the 7:35 and 35:7 diets were excluded from the analysis, $F_{1,77}$ (P)=3.7 (0.058) for the effect of phase; $F_{2,77}$ (P)=21.6 (<0.001) for diet treatment and $F_{2,77}$ (P)=1.057 (0.352) for the diet by phase interaction]. On diet 7:35, gregarious insects deposited more lipid than did solitarious locusts, but this was simply the result of their having eaten more carbohydrate.

Performance consequences

Solitarious locusts developed more quickly than did gregarious insects on all diets except for the two extremes, 7:35 and 35:7, where stadium duration did not differ (Fig. 5) [$F_{1,114}$ (P)=6.26 (0.014) for the effect of phase on development time; Lifereg Procedure, i^2_1=10.29, P=0.001]. The faster development of solitarious locusts on more balanced diets was associated with their higher efficiency of conversion of ingested nutrients (particularly protein) to growth (Fig. 4), while their extended development on the two extreme diets resulted from

having eaten less of these foods (Figs 1, 2). Although they developed more slowly on more balanced diets, gregarious nymphs showed less of an effect of dietary imbalance on development time as a result of having eaten more of the imbalanced diets than did solitarious insects (Figs 1, 2).

None of the solitarious locusts died during the experiment, whereas gregarious nymphs survived less well on diets containing excess carbohydrate and a deficit of protein, notably diet 7:35 (Fig. 5).

Discussion

Our data indicate that, in addition to other known phase-related differences in behaviour, colour, morphometry and physiology, solitarious and gregarious desert locust nymphs exhibit contrasting nutritional strategies. While the two phases had similar intake requirements for protein and carbohydrate, they differed markedly in their strategies for procuring these nutrients when faced with nutritional imbalance. In addition, they differed in the efficiency with which they converted ingested nutrients into growth and in the performance consequences of ingesting nutritionally unbalanced foods.

Position of the intake target

Both phases of locust regulated their intake of protein and digestible carbohydrate to a statistically indistinguishable point when challenged with two different pairings of complementary, unbalanced foods. In the present experiment, we only used two food pairings, but the results indicating homeostatic intake are fully consistent with our earlier work with fifth-instar nymphs of *Locusta migratoria*, which maintained protein and carbohydrate intake in the face of four complementary food pairings (Chambers et al., 1995), a fivefold dietary dilution with cellulose (Raubenheimer and Simpson, 1993) and alteration of the relative frequency with which complementary foods occurred in the environment (Behmer et al., 2001).

That there were no phase-associated differences in protein–carbohydrate intake is, in one respect, unexpected, given the difference in activity levels and metabolic rate between the two phases (Pener, 1991; Simpson et al., 1999). However, in the present experiments, the nymphs were kept individually in arenas with the two dishes of complementary food within a close distance, thus requiring little locomotion. Also, being kept alone induces partial behavioural solitarisation in gregarious nymphs (Roessingh and Simpson, 1994), such that they become less active. It is likely that adults would not have the same protein–carbohydrate intake requirements in the solitarious and gregarious phases, given their very different strategies for lipid metabolism associated with flight and migration (Pener et al., 1997).

Balancing nutrient intake: the nutritional heterogeneity hypothesis

When plotted as nutrient intake arrays, the pattern for solitarious nymphs was closely similar to that described

previously for gregarious *L. migratoria* (Raubenheimer and Simpson, 1993): a pattern we have termed the 'closest distance rule' since it represents the behavioural solution that minimises nutritional error relative to the intake target. Gregarious *S. gregaria*, in contrast, ingested more of the nutrient in excess in unbalanced foods and, hence, suffered less of a deficit in intake of the limiting nutrient: they behaved as nutrient maximisers rather than error minimisers (Raubenheimer and Simpson, 1997). There has been one earlier report of crowd-reared *S. gregaria* eating more than those reared in isolation (Norris, 1961), but the effects of phase *per se* could not be distinguished from the excitatory influence of being in a crowd (see Simpson et al., 1988).

Interestingly, the transition between the intake patterns for the two phases appears to take more than one generation to complete. One-generation solitary-reared nymphs were intermediate between gregarious and three-generation solitarious nymphs: they had developed the solitarious pattern of eating less on diet 7:35, but this was not the case for the oppositely unbalanced diet, 35:7.

The differences between the two phases of *S. gregaria* in their strategies for balancing nutrient intake are consistent with the contrasting nutritional environments of the two phases, according to the following hypothesis. The amount of a nutritionally unbalanced food that an animal eats ought to reflect the probability that it will subsequently encounter an equally and oppositely unbalanced food (Raubenheimer and Simpson, 1999). If this probability is high, then the animal should eat a large amount, since the cost of over-ingesting the nutrients in excess in the first food will be mitigated by eating the complementary food. Indeed, the excess nutrient from the first food will be translated into useful nutriment, yielding a greater total intake of a balanced diet than if only a small amount had been eaten. In contrast, if the probability of encountering an oppositely unbalanced food in the future is low, then eating a large amount of an unbalanced food will potentially have greater fitness costs than would accrue from eating a smaller amount and using it for growth with high efficiency. In short, a trade-off is predicted between maximising intake of a balanced diet and minimising nutritional error. The solution to this trade-off will depend on the distribution of foods of different nutritional composition in an animal's environment and the frequency with which these foods are encountered and accepted. A corollary of this hypothesis is that were an animal that is adapted to eat large amounts of unbalanced foods, with the functional expectation of encountering complementary foods in the future, to be restricted to a single sub-optimal food, it should suffer the costs of having over-ingested the excess nutrients.

Two factors will influence the probability of encountering complementary food. The first is the spatial and temporal distribution of foods of differing nutritional composition in the environment, and the second is the behaviour of the animal, notably its mobility and the sensory criteria it uses for accepting food. Mobility will, in turn, reflect other features of the environment, in particular the extent to which the risks from natural enemies are increased while moving (Bernays, 1997).

Solitarious nymphs of the desert locust are far less mobile than their gregarious counterparts, as befits their low population density and cryptic lifestyle (Uvarov, 1977; Pener, 1991; Simpson et al., 1999). Also, although they are potentially highly polyphagous, solitarious nymphs typically live in environments with few host-plant species (Ghaout et al., 1991). As a result of being less mobile and living in a florally impoverished environment, it seems likely that, on average, they will experience less nutritional heterogeneity than will gregarious nymphs, leading us to predict that solitarious nymphs should tend towards minimising nutritional error rather than maximising nutrient intake. Local food-plant specialisation is also a feature of certain New World species of *Schistocerca*, which do not show the marked phase change seen in *S. gregaria* (Chambers et al., 1996; Sword and Chapman, 1994; Sword and Dopman, 1999). It would be extremely interesting to establish how these compare with solitarious and gregarious *S. gregaria* in their nutrient balancing strategies.

Why then, should gregarious-phase nymphs of the African migratory locust *L. migratoria* exhibit a closely similar pattern of nutrient balancing to solitarious *S. gregaria*, rather than employing the same strategy as gregarious-phase *S. gregaria*? Such an outcome would be expected if solitarious-phase *S. gregaria* and gregarious-phase *L. migratoria* both experience less nutritional heterogeneity than do gregarious-phase *S. gregaria*. It is relevant here that *L. migratoria* is restricted to feeding on grasses, although the actual range and frequency of nutritional compositions encountered in the field by these (or indeed any other) insect herbivores have yet to be measured.

The 'nutritional heterogeneity' hypothesis provides a possible functional explanation for the observed differences in intake pattern between solitarious and gregarious *S. gregaria*, but there are other potential explanations. Perhaps gregarious nymphs eat more of the nutritionally imbalanced foods (or maybe of any less suitable food) as an adaptation to living under crowded conditions in degrading habitats, where there is competition for scarce, poor-quality food. However, this should also be expected for gregarious *L. migratoria*, which form similarly large aggregations, yet this species exhibits similar nutritional responses to solitarious *S. gregaria*.

Another possibility is that the difference in consumption of unbalanced foods reflects differences in physiological needs of the two phases. Phase-related differences in behaviour and physiology clearly exist (for a review, see Pener, 1991), but the fact that the self-selected intake of protein and carbohydrate did not differ between the phases (see above) suggests that this is not the reason for the observed patterns of nutrient balancing in the present study. However, perhaps gregarious nymphs were more active than solitarious nymphs when confined to a single, sub-optimal food, and the associated energetic demands led to a need for gregarious nymphs to ingest greater amounts of unbalanced foods than did solitarious nymphs. We did not measure levels of locomotion in the present study, but the

restricted size of the arenas and the low metabolic costs of walking within them, as measured for locust nymphs using flow-through respirometry (Zanotto et al., 1997; Gouveia et al., 2000), make this an unlikely explanation.

Nutrient utilisation

The most striking difference in nutrient utilisation between the phases was that solitarious nymphs regulated body protein growth to a higher level than did gregarious locusts, despite both having eaten similar amounts of protein across the stadium. Thus, gregarious insects converted ingested protein to growth with lower efficiency. Providing that nymphs ingested more than approximately 0.2 g across the stadium (which was the case on all diets, except for the food with lowest protein level, 7:35), protein intake did not influence protein growth in either phase. Regulation of body protein content in *L. migratoria* has been shown to result from a high efficiency of conversion to growth for amounts of protein eaten that are less than the intake target level, then removal of excess ingested protein through nitrogenous excretion (Zanotto et al., 1993). It is possible that the extra protein (relative to solitarious insects) that was eaten but not used for growth in gregarious locusts was deaminated and the carbon skeletons used for energetic and structural purposes (Thompson, 1998).

Solitarious locusts showed a tendency to convert more ingested carbohydrate to body lipid on the more balanced diets than did gregarious nymphs. This, however, is probably because gregarious nymphs took longer to develop and hence had to maintain themselves for longer.

Performance consequences

Solitarious locusts developed faster than did gregarious nymphs across all but the most extremely unbalanced foods (7:35 and 35:7). On these latter two diets, stadium duration did not differ between the phases. This resulted from the need for solitarious insects to prolong development to make up their intake of the deficient nutrient and is indicated by the straightening of the intake array seen in Fig. 1. Gregarious insects had less need to extend development on the extreme foods, since their intake of unbalanced foods was consistently greater throughout the stadium. Slower nymphal development rates for gregarious than solitarious *S. gregaria* have been reported previously [P. Hunter-Jones, unpublished results (cited in Uvarov, 1966)].

As mentioned above, it might be expected that ingesting excess nutrient, then having to remove it, would have accompanying fitness costs, both metabolic and ecological (Bernays, 1997). Consistent with this prediction, gregarious nymphs survived less well than solitarious insects when provided with a food containing high carbohydrate and low protein levels. This was the dietary regime on which gregarious locusts over-ate most relative to solitarious insects. For intakes in excess of approximately 0.3 g across the stadium, both phases converted carbohydrate to lipid at a rate of 0.25 g lipid deposited per gram of carbohydrate eaten [a similar rate to

that reported previously for *L. migratoria* (Simpson and Raubenheimer, 2001)]. As a result, gregarious insects on diet 7:35 deposited 102 mg of lipid (representing 30 % of the total body dry mass of 339 mg for insects on the 7:35 treatment) compared with 72 mg (22 % of 321 mg total body mass) in solitarious insects. Whether this difference in body lipid contributed to the higher mortality of gregarious nymphs is not known, but the possibility of morbid obesity in insects is an intriguing thought.

Differences have been reported among other grasshopper species in survivorship, growth and fecundity when subjected to experimental manipulation of their dietary carbohydrate and protein content (Joern and Behmer, 1997, 1998). Ecologically linked differences between species in the position of the intake target and strategies of nutrient balancing would account for such effects.

A broad aim of our research has been to identify general principles underlying differences in the nutritional strategies of animals and key sites for the action of selection in the evolution of those strategies. We have demonstrated marked differences in nutritional regulatory responses between the phases of the desert locust and provided possible functional explanations for these differences. As further data emerge from other systems, the veracity and generality of our nutritional heterogeneity hypothesis can be tested using comparative analysis. Another aim is to test such ecological hypotheses in selection experiments in which the range, mean and variance of nutritional compositions is experimentally varied and the effects on patterns of nutrient regulation and utilisation are measured.

References

Behmer, S. T., Raubenheimer, D. and Simpson, S. J. (2001). Frequency-dependent food selection in locusts: a geometric analysis of the role of nutrient balancing. *Anim. Behav.* **61**, 995–1005.

Bernays, E. A. (1997). Feeding by lepidopteran larvae is dangerous. *Ecol. Entomol.* **22**, 121–123.

Bouaïchi, A., Simpson, S. J. and Roessingh, P. (1996). The influence of environmental microstructure on the behavioural phase state and distribution of the desert locust *Schistocerca gregaria*. *Physiol. Entomol.* **21**, 247–256.

Chambers, P. G., Simpson, S. J. and Raubenheimer, D. (1995). Behavioural mechanisms of nutrient balancing in *Locusta migratoria* nymphs. *Anim. Behav*. **50**, 1513–1523.

Chambers, P. G., Sword, G., Angel, J. E., Behmer, S. and Bernays, E. A. (1996). Foraging by generalist grasshoppers: two different strategies. *Anim. Behav.* **56**, 155–165.

Collett, M., Despland, E., Simpson, S. J. and Krakauer, D. C. (1998). Spatial scales of desert locust gregarization. *Proc. Natl. Acad. Sci. USA* **95**, 13052–13055.

Despland, E., Collett, M. and Simpson, S. J. (2000). Small-scale processes in desert locust swarm formation: how vegetation patterns influence gregarization. *Oikos* **88**, 652–662.

Despland, E. and Simpson, S. J. (2000a). The role of food distribution and nutritional quality in behavioural phase change in the desert locust. *Anim. Behav.* **59**, 643–652.

Despland, E. and Simpson, S. J. (2000b). Small-scale vegetation patterns in

the parental environment influence the phase state of hatchlings of the desert locust. *Physiol. Entomol.* **25**, 74–81.

Ellis, P. E. (1951). The marching behaviour of hoppers of the African migratory locust (*Locusta migratoria migratorioides* R. and F.) in the laboratory. *Anti-Locust Bull.* **7**, 1–46.

Ghaout, S., Louveaux, A., Mainguet, A. M., Deschamps, M. and Rahal, Y. (1991). What defense does *Schouwia purpurea* (Cruciferae) have against the desert locust? Secondary compounds and nutritive value. *J. Chem. Ecol.* **17**, 1499–1516.

Gouveia, S. M., Simpson, S. J., Raubenheimer, D. and Zanotto, F. P. (2000). Patterns of respiration in *Locusta migratoria* nymphs when feeding. *Physiol. Entomol.* **25**, 88–93.

Hägele, B. F. and Simpson, S. J. (2000). The influence of mechanical, visual and contact chemical stimulation on the behavioural phase state of solitarious desert locusts (*Schistocerca gregaria*). *J. Insect Physiol.* **46**, 1295–1301.

Jablonka, E. and Lamb, M. J. (1998). Epigenetic inheritance in evolution. *J. Evol. Biol.* **11**, 159–183.

Joern, A. and Behmer, S. T. (1997). Importance of dietary nitrogen and carbohydrates to survival, growth and reproduction in adults of the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae). *Oecologia* **112**, 201–208.

Joern, A. and Behmer, S. T. (1998). Impact of diet quality on demographic attributes in adult grasshoppers and the nitrogen limitation hypothesis. *Ecol. Entomol.* **23**, 174–184.

Kennedy, J. S. (1939). The behaviour of the desert locust (*Schistocerca gregaria* (Forsk.)) (Orthopt.) in an outbreak center. *Trans. R. Entomol. Soc. Lond.* **89**, 385–542.

McCaffery, A. R., Simpson, S. J., Islam, M. S. and Roessingh, P. (1998). A gregarizing factor present in the egg pod foam of the desert locust *Schistocerca gregaria*. *J. Exp. Biol.* **201**, 347–363.

Mousseau, T. A. and Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends Ecol. Evol.* **10**, 403–407.

Norris, M. J. (1961). Group effects on feeding in adult males of the Desert Locust, *Schistocerca gregaria* (Forsk.) in relation to sexual maturation. *Bull. Ent. Res.* **51**, 731–753.

Pener, M. P. (1991). Locust phase polymorphism and its endocrine relations. *Adv. Insect Physiol.* **23**, 1–79.

Pener, M. P., Ayali, A. and Golenser, E. (1997). Adipokinetic hormone and flight fuel related characteristics of density-dependent locust phase polymorphism: a review. *Comp. Biochem. Physiol.* **117B**, 513–524.

Pener, M. P. and Yerushalmi, Y. (1998). The physiology of locust phase polymorphism: an update. *J. Insect Physiol.* **44**, 365–377.

Raubenheimer, D. and Simpson, S. J. (1993). The geometry of compensatory feeding in the locust. *Anim. Behav.* **45**, 953–964.

Raubenheimer, D. and Simpson, S. J. (1994). The analysis of nutrient budgets. *Funct. Ecol.* **8**, 783–791.

Raubenheimer, D. and Simpson, S. J. (1997). Integrative models of nutrient balancing: application to insects and vertebrates. *Nutr. Res. Rev.* **10**, 151–179.

Raubenheimer, D. and Simpson, S. J. (1999). Integrating nutrition: a geometrical approach. *Entomol. Exp. Appl.* **91**, 67–82.

Roessingh, P., Bouaïchi, A. and Simpson, S. J. (1998). Effects of sensory stimuli on the behavioural phase state of the desert locust, *Schistocerca gregaria*. *J. Insect Physiol.* **44**, 883–893.

Roessingh, P. and Simpson, S. J. (1994). The time-course of behavioural phase change in nymphs of the desert locust, *Schistocerca gregaria*. *Physiol. Entomol.* **19**, 191–197.

Roessingh, P., Simpson, S. J. and James, S. (1993). Analysis of phase-related changes in the behaviour of desert locust nymphs. *Proc. R. Soc. Lond. B* **252**, 43–49.

Roffey, J. and Popov, G. (1968). Environmental and behavioural processes in a desert locust outbreak. *Nature* **219**, 446–450.

Simpson, S. J. and Abisgold, J. D. (1985). Compensation by locusts for changes in dietary nutrients: behavioural mechanisms. *Physiol. Entomol.* **10**, 443–452.

Simpson, S. J., Despland, E., Hägele, B. F. and Dodgson, T. (2001). Gregarious behavior in desert locusts is evoked by touching their back legs. *Proc. Natl. Acad. Sci. USA* **98**, 3895–3897.

Simpson, S. J., McCaffery, A. R. and Hägele, B. F. (1999). A behavioural analysis of phase change in the desert locust. *Biol. Rev.* **74**, 461–480.

Simpson, S. J. and Raubenheimer, D. (1993). A multi-level analysis of feeding behaviour: the geometry of nutritional decisions. *Phil. Trans. R. Soc. Lond. B* **342**, 381–402.

Simpson, S. J. and Raubenheimer, D. (2000). The hungry locust. *Adv. Study Behav.* **29**, 1–44.

Simpson, S. J. and Raubenheimer, D. (2001). The geometric analysis of nutrient–allelochemical interactions: a case study using locusts. *Ecology* **82**, 422–439.

Simpson, S. J., Simmonds, M. S. J., Wheatley, A. R. and Bernays, E. A. (1988). The control of meal size in the locust. *Anim. Behav.* **36**, 1216–1227.

Sword, G. A. (1999). Density-dependent warning coloration. *Nature* **397**, 217.

Sword, G. A. and Chapman, R. F. (1994). Monophagy in a polyphagous grasshopper. *Entomol. Exp. Appl.* **73**, 255–264.

Sword, G. A. and Dopman, E. B. (1999). Developmental specialization and geographic structure of host plant use in a polyphagous grasshopper, *Schistocerca emarginata* (=*lineata*) (Orthoptera: Acrididae). *Oecologia* **120**, 437–445.

Sword, G. A., Simpson, S. J., Taleb, O., El Hadi, M. and Wilps, H. (2000). Density-dependent aposematism in the desert locust. *Proc. R. Soc. Lond. B* **267**, 63–68.

Thompson, S. N. (1998). Long-term regulation of glucogenesis by dietary carbohydrate and relevance to blood sugar level in an insect *Manduca sexta*. *Int. J. Biochem. Cell Biol.* **30**, 987–999.

Uvarov, B. (1966). *Grasshoppers and Locusts*, vol. I. Cambridge: Cambridge University Press.

Uvarov, B. (1977). *Grasshoppers and Locusts*, vol. II. London: Centre for Overseas Pest Research.

Zanotto, F. P., Gouveia, S. M., Simpson, S. J., Raubenheimer, D. and Calder, P. C. (1997). Nutritional homeostasis in locusts: is there a mechanism for increased energy expenditure during carbohydrate overfeeding? *J. Exp. Biol.* **200**, 2437–2448.

Zanotto, F. P., Simpson, S. J. and Raubenheimer, D. (1993). The regulation of growth by locusts through post-ingestive compensation for variation in the levels of dietary protein and carbohydrate. *Physiol. Entomol.* **18**, 425–434.