



Integrating nutrition: a geometrical approach

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Accepted: October 18, 1998

Key words: nutritional theory, nutrient balancing, locusts, protein intake, carbohydrate intake, salt intake

Abstract

We present and illustrate using data from insects an integrative approach to modelling animal nutrition. This framework enables the unification within simple geometrical models of several nutritionally relevant measures. These include: the optimal balance and amounts of nutrients required to be ingested and allocated to growth by an animal over a given time period (the intake and growth targets, respectively); the animal's current state in relation to these requirements; available foods and the consequences for the animal's state of ingesting them; the amounts of ingested nutrients that are retained and eliminated; and animal performance. Data are presented on intake targets in insects, illustrating how they change over various time-scales (physiological, developmental, and evolutionary). Most importantly, the geometrical approach enables a clear description to be made of the trade-offs reached by animals in regulating their nutritional balance. Animals given a nutritionally balanced food, or two or more imbalanced but complementary foods, can satisfy their nutrient requirements. However, those eating non-complementary imbalanced foods must reach a suitable compromise between over-ingesting some nutrients and under-ingesting others. Data are presented comparing the rules of compromise for protein and carbohydrate ingestion in grass-feeding (*Locusta migratoria*) and polyphagous (*Schistocerca gregaria*) locusts.

Introduction

A recurring theme in the study of animal behaviour has been integration. This has developed largely on two fronts. First, there have been repeated exhortations that an aim of ethologists should be to work towards an understanding of behaviour that integrates across different *levels of analysis*. Following Tinbergen (1951, 1963) four levels, or some variants thereof, are usually recognised: the mechanisms, the development, the evolutionary history and the functions of behaviour. See Dawkins (1989) and Dewsbury (1992) for further discussion of the levels of analysis in behavioural studies.

A second field of study where integration has been a primary aim is in attempts to understand behaviour as the outcome of a multiplicity of interacting causes. This is, in other words, an attempt to understand how animals integrate causal factors to produce behaviour that is appropriate to a given circumstance. For instance, a foraging animal detecting some signs

of potential predators must decide whether to flee or to feed; the resulting behaviour is contingent on the integration of the external (e.g., strength of predator and food cues) and internal (nutritional state, history of interactions with predators, etc.) factors. The major approaches to studying such *organismal integration* have been the use of control theory to model the interactions among physiology, behaviour and the animal's external environment (e.g., Calow, 1976; Toates, 1986), and the development of state space models (McFarland & Houston, 1981).

Among the categories of animal behaviour, nutrition poses particular challenges to workers wishing to achieve an integrative understanding of their subject. The above issues of the levels of analysis and the organismal integration of nutritional decisions with other behavioural systems are of course relevant. In addition, the process of nutritional regulation is itself a complex challenge of integration for the organism. Firstly, animals usually require several nutrients simultaneously, at individual levels that may change

with recent nutritional history, developmental stage, levels of activity, etc. The various requirements are met by eating foods which might seldom contain the nutrients at the required levels – and which are, furthermore, frequently (e.g., noxious allelochemicals deleterious synthesized by plant tissue). Secondly, in its passage from the environment to the anus, there are various levels at which a food is processed to minimise the discrepancy between its chemical composition and the nutritional requirements of the animal (e.g., habitat selection, food selection, food switching, and the regulation of absorption, assimilation and excretion). There are several impressive examples of how animals integrate the various stages of nutritional processes, those closest to home concerning locusts (e.g., Simpson et al., 1995; Simpson & Raubenheimer, 1993a, b, 1996; Raubenheimer & Simpson, 1993, 1996, 1997, 1998).

Over the past few years we have developed and applied to the study of insects and vertebrates a powerful framework for integrative research into nutritional processes (Raubenheimer & Simpson, 1993, 1994, 1997; Simpson & Raubenheimer, 1993a, 1997; Simpson et al., 1995). The framework is centred on simple geometrical models that enable the consideration in a single scheme of all of Tinbergen's levels of analysis, as well as various categories of organismal integration, including the issues of multiple nutrients and the serial stages of nutritional processing. Here we provide an overview of our approach, developing the key concepts and presenting data illustrating their application to insect nutrition.

Intake requirements

The need for an integrative approach. A central reference point to our explorations of nutritional processes is the ingestive requirements of the animal. Hitherto, there have been three major approaches to characterising these requirements. The food-centred approach considers food types the relevant currency of animal feeding behaviour (Figure 1a). While this approach is important in understanding ecological processes, on its own it has severe limitations in organism-centred studies: foods are merely the vehicles to animals for the nutrients they contain. Therefore, the primary target of natural selection is at the level of nutrients, and any patterns concerning food intake are most readily explicable at this level.

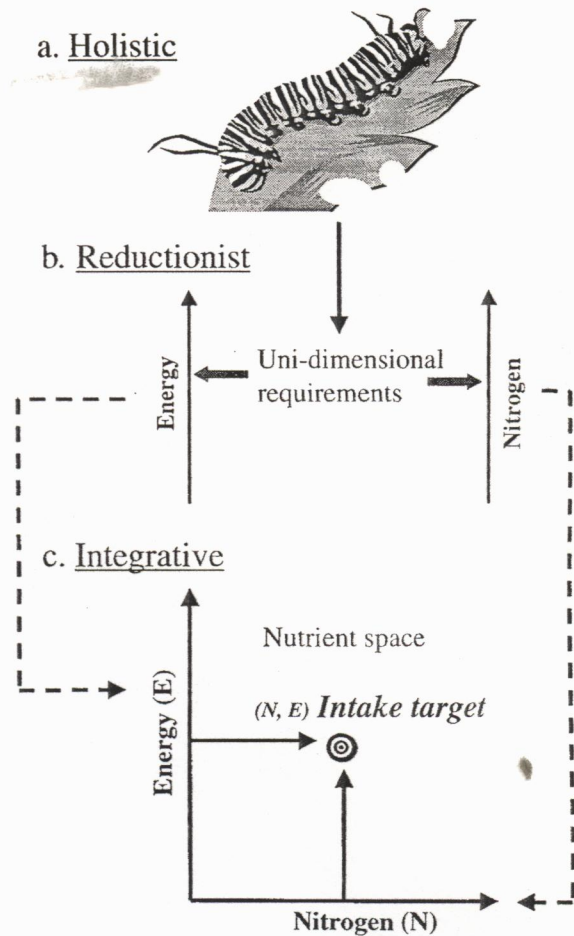


Figure 1. Three approaches to characterising nutritional requirements.

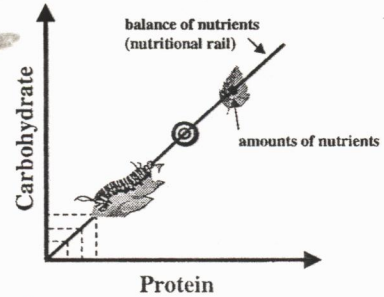
Nutrient-centred approaches have developed along two lines (Figure 1b). The one, most explicitly articulated in the optimal foraging paradigm (Stephens & Krebs, 1986), considers energy (i.e., energy-yielding nutrients) the primary currency. An elaboration of this approach has been to consider non-energetic nutrients, only in so far as they impose constraints on energy intake (Pulliam, 1975; Belovsky, 1990). The other, most prevalent in the literature on the nutritional ecology of insects, considers nitrogen as primary (McNeill & Southwood, 1978; Mattson, 1980; White, 1993). While both approaches have yielded impressive insights, they are limited in generality since a single nutrient will constitute a strong predictor of behaviour only under a restricted range of circumstances. For instance, animals undergoing somatic or reproductive growth might prioritise the intake of nitrogen, and those feeding predominately to fuel en-

ergy metabolism would weight more strongly energy intake. For those feeding on nutritionally balanced foods, energy and nitrogen can be considered a single resource and therefore either or both will predict behaviour equally (see below).

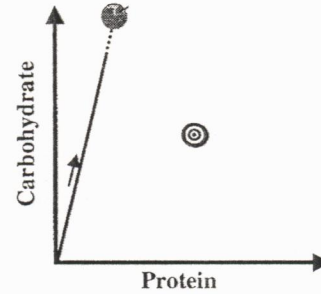
A major limitation of the single-nutrient approach is therefore that it deals only peripherally, or not at all, with the central nutritional question of how animals integrate the intake of various nutrients. There are, however, at least two *a-priori* reasons why nutritional integration should be considered a central issue in attempts to understand animal nutrition. Firstly, even though a single nutrient might well be the most limiting at a given time, in most instances animals simultaneously require other nutrients. Growing animals, for example, require energy to fuel metabolic requirements, while non-growing ones require a source of nitrogen for enzyme synthesis, tissue repair and so forth. Secondly, the single nutrient approach places undue emphasis on the deficient nutrient being the 'limiting' one. However, since animals (and particularly herbivores) seldom feed on foods that are perfectly balanced nutritionally, they frequently are forced to ingest excesses of other nutrients in order to limit the shortfall of the deficient one (Raubenheimer, 1992; Simpson & Raubenheimer, 1993a; Raubenheimer & Simpson, 1993). The ability to ingest surplus quantities of excessive nutrients might thus be an important limitation on animals – indeed, our research suggests that for some insects and vertebrates this might be a primary limiting factor in nutritional regulation (Raubenheimer & Simpson, 1997). An adequate explanation of nutritional behaviour should thus incorporate not only the deficient nutrient, but also the excessive ones.

The geometrical approach. Our approach has therefore been to combine the axes representing various nutrients into one (or more – see below) two-dimensional *nutrient spaces* (Figure 1c). In this nutrient space appears a point representing the individual amounts of the various nutrients (and hence their balance) that should optimally be ingested by the animal over a given time, the *intake target*. Foods too can be depicted in the nutrient space, and so can the relationship between a given food item and the animal's current requirements. For example, the leaf in Figure 2a is defined in the scheme by the amount it contains of the two nutrients, both of which are present in quantities that exceed the respective target co-ordinates. Inherent in this description is a further, critical, parameter

a. Balanced food



b. Imbalanced food



c. Complementary foods

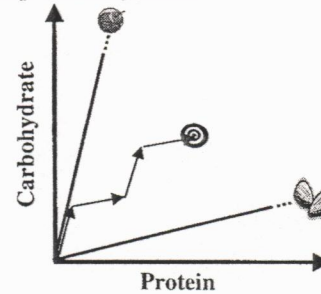


Figure 2. The amounts of nutrients in a food item can be represented as a point in nutrient space (e.g., the leaf in a.), while the balance of nutrients is described by a linear trajectory (nutritional rail) that project into a nutrient space at an angle describing the ratio of nutrients. If this ratio is the same as is required by an animal (i.e., it is a nutritionally balanced food), the animal's intake target can be reached by feeding on the food (a). It cannot, by contrast, be reached by feeding on an imbalanced food (b). It can, however, be reached by switching between two complementary imbalanced foods (c). In b. and c. (and subsequent figures) the foods are described only by their balance of nutrients (nutritional rails), since the amounts of nutrients in each are assumed to exceed the scale of the axes.

describing the leaf, the balance of the two nutrients it contains. This is depicted as a linear trajectory from the origin to the point describing the amounts of the nutrients in the leaf. Such trajectories representing foods have been called *nutritional rails*, to capture the fact that as the animal eats this food its nutritional state changes along a path coincident with this trajectory (Raubenheimer & Simpson, 1993). In this case, the nutritional rail passes through the intake target, and the

animal's nutritional requirements can therefore be satisfied by eating the food. This leaf is, in other words, an adequate food in two respects: it contains sufficient quantities of both nutrients to satisfy the animal's requirements, and the nutrients are balanced with respect to these requirements.

The rail in Figure 2b, by contrast, describes a nutritionally imbalanced food, containing carbohydrates in excessive concentration relative to proteins. This rail does not pass through the intake target, and an animal with access only to this food is thus unable to satisfy its optimal nutrient requirements. The options open to an animal in this predicament are discussed in some detail below.

The animal could, however, reach the intake target if in addition to this food it had available a second nutritionally imbalanced food, provided the rail describing the second food lay on the opposite side of the intake target to the first (i.e., containing an excess of protein relative to carbohydrate). As shown in Figure 2c, this could be done by switching between the foods, and thus moving alternately along a trajectory parallel with the one then the other of the rails. Combinations of nutritionally imbalanced foods that allow an animal to 'zigzag' its way through nutrient space in this manner to the intake target are termed *complementary* foods.

Experimentally locating the intake target. Since our approach is primarily an empirical one, it is necessary to give due consideration to obtaining data on the position of intake targets [see Simpson & Raubenheimer (1995) for further discussion on such methodological issues]. An important step in estimating this position is to measure the free-running output of the regulatory mechanisms for the nutrients in consideration. The assumption here is that nutritional regulatory mechanisms have been configured by natural selection to ensure that when the environment permits they provision the animal with the optimal amounts and balance of nutrients. Experimentally, this approach requires that the animal has available complementary foods whose nutritional rails jointly span a sufficiently large area in nutrient space to encompass the intake target, as shown in Figure 2c. The point actually selected by the animal should, by this reasoning, provide a good estimate of the position of the target.

An important consideration in planning such studies, is the nature of the foods to be used. There exists, potentially, a trade-off between the ecological realism achieved using natural foods, and the analytical power

available using synthetic foods. While much of our work to date has used chemically defined synthetic foods [see Simpson & Abisgold (1985) and Raubenheimer & Simpson (1993) for details of these foods], recent work in the field (Wright, 1998; G. A. Wright, S. J. Simpson & D. Raubenheimer, unpubl.) and using genetically modified *Arabidopsis* mutants (Wright, 1998; G. A. Wright, D. Raubenheimer & S. J. Simpson, unpubl.) illustrate the power of the geometrical approach when applied to more complex systems. For clarity, the illustrative data presented in this paper all concern synthetic foods, in which the number of relevant nutritional dimensions can readily be experimentally controlled. In the final part of this paper we discuss some important considerations in expanding the models to deal with a greater number of nutritional dimensions.

Whatever the number of dimensions, there are some caveats to heed in using the above approach to identifying the position of intake targets. Firstly, the foods used should not be so extreme in their imbalance that they are not recognised by the animal as food (*pathological foods*), but at the same time should be sufficiently divergent to provide a high probability that they encompass the target position. Fortunately, first estimates of the positions of the intake target rail are available for a wide range of insect species (Simpson & Raubenheimer, 1993a; see also below), and this should enable the selection of moderately divergent experimental foods in most cases. Furthermore, a good indicator that inappropriate rails have been used is obtained when an animal takes a substantial majority of its intake from one of the foods. Most likely, in this case the target lies outside of the accessible area of nutrient space, on the side of the favoured food (Chambers et al., 1995, 1997).

A second caveat is that in order to have some degree of confidence that a selected point in nutrient space does represent the intake target, it is necessary to demonstrate that this is the outcome of nutritional regulation rather than the result of some process independent of the nutrients in consideration (e.g., random feeding on the two foods). This is achieved by including in the experiment several food pairings, such that the animals in the different treatment groups would have to alter the proportions of the two foods ingested to achieve the same point in nutrient space. Such *target defence* was demonstrated for protein and carbohydrate regulation in *Locusta migratoria* by using treatment pairings in which one of the foods, both foods or neither of the foods were diluted with in-

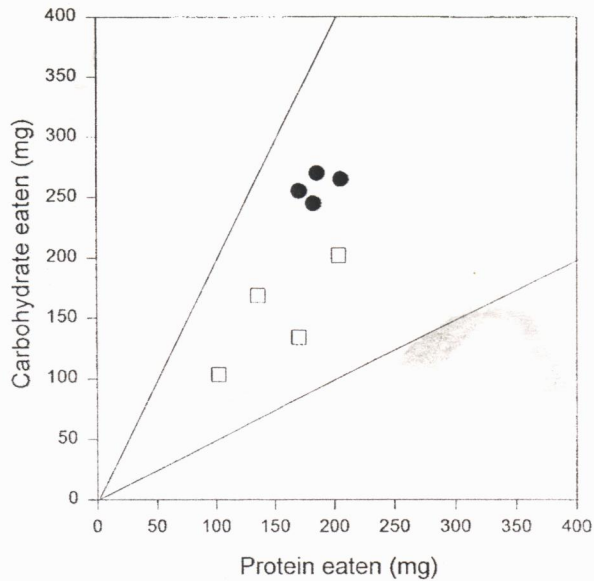


Figure 3. Selected intake points of *L. migratoria* over the first 6 days of the 5th stadium. Circles represent observed two-dimensional means of four treatments ($n = 10$) given food pairings (%protein:%carbohydrate) 28 : 14 + 14 : 28; 28 : 14 + 7 : 14; 14 : 7 + 14 : 28; 14 : 7 + 7 : 14. Squares represent hypothetical outcome in the absence of target defence.

digestible cellulose bulk (Figure 3) Chambers et al., 1995. Where it is not feasible to use diluents, an alternative approach would be to use one more- and one less-extreme rail on either side of the suspected target position. Treatments would then consist of complementary pairings either of the two more-extreme rails, the two moderate rails or one of each (e.g., see Trumper & Simpson, 1993).

Thirdly, it is useful to ensure that the foods used in each treatment pairing are not too dissimilar in respects other than the nutrients of interest. Otherwise, there is a danger that the proportion of the two foods ingested (hence the point achieved in nutrient space) represents regulation not only for the nutrients in question, but also for other constituents. This is a further context in which experiments to test defence of the target, as described above, are useful. If animals regulate to the same point in, for example, a protein-carbohydrate nutrient space despite the fact that the relative levels of other nutrients have been altered, then this suggests very strongly that there are no hidden nutritional dimensions influencing the results.

Finally, an important source of subsidiary information on the position of intake targets comes from performance measures. Intake targets are functional

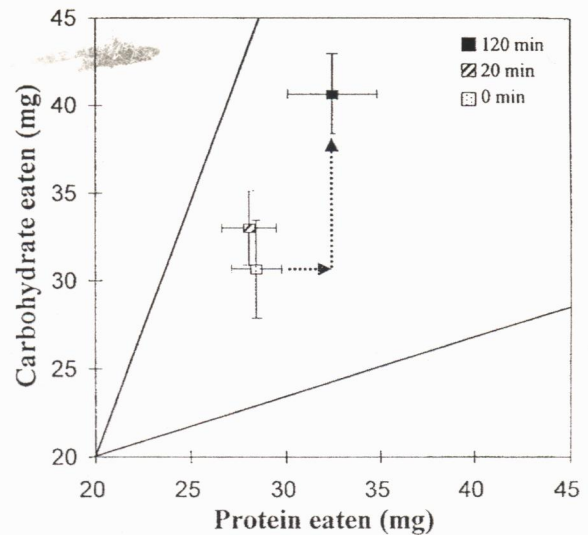


Figure 4. Nutrient selection by adult female *L. migratoria* given foods 14:28 and 28:14 (% protein:% digestible carbohydrate) for 24 h after flying for varying periods.

optima, so a situation where animals show poor performance across a number of fitness measures at the selected point of intake (relative to groups experimentally confined to foods that restrict them to elsewhere in nutrient space) suggests that confounding factors are operative. For example, the food pairings might not encompass the target rail, pathological foods might be involved, and/or there might be important dimensions that are omitted from the model. On the other hand, performance at the intake target need not be optimal by all measures, since animals might be naturally selected to occupy positions in nutrient space that represent a favourable balance among conflicting nutrient requirements and ecological pressures. An example of this is presented below.

Intake targets are dynamic

While intake targets provide a central reference point in our geometrical models of nutrition, it is important to emphasise that this is a dynamic, rather than static reference point. There are, in fact, several levels at which targets move, including across physiological, developmental, and evolutionary time-scales.

The optimal nutrient requirements at a given time depend on the physiological demands that are placed on the animal, and the regulation of nutrient intake should reflect this. Figure 4 shows, for example, the selected intake points by adult *Locusta migratoria*

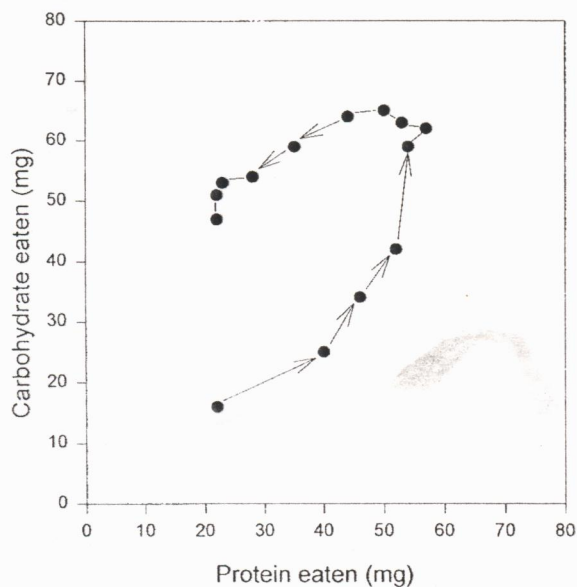


Figure 5. Changes in daily intake of protein and carbohydrate in adult *L. migratoria* allowed to select between foods from the time of moulting until the end of the somatic growth phase.

flown for 20 min, 120 min and unflown controls (D. Raubenheimer & L. Firth, unpubl.). Although there was no appreciable difference in the selected point for control insects and those flown for 20 min, those flown for 120 min selected a substantially different point. Most interesting is that the movement of the target following 120 min flight was significant along the carbohydrate axis, but not the protein axis. Presumably, this reflects the fact the energy-providing role of carbohydrates in these insects (Jutsum & Goldworthy, 1976).

On a longer time-scale, intake targets move with development, reflecting the different nutrient requirements of various stages of growth, reproduction and senescence. Figure 5 for example shows the changes in daily intake of protein and carbohydrate in adult female *Locusta migratoria* allowed to switch between foods from the time of moulting until the end of the somatic growth phase (data from Chyb & Simpson, 1990). Total intake increased for the first four days following the moult, but the ratio of protein to carbohydrate remained fairly constant. On the fifth day, the amount of protein ingested began to decrease relative to carbohydrate. On subsequent days this relative decrease continued, and the total amounts of each nutrient eaten also declined, as is evident from the backwards loop of the target trajectory. This relative decrease in protein intake, and the absolute decrease

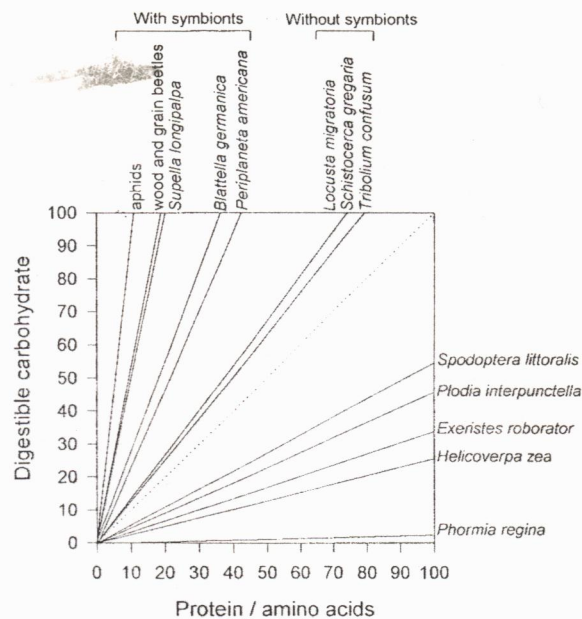


Figure 6. Comparison of the optimal dietary protein:carbohydrate ratio of various insect groups. Insects with the lowest protein:carbohydrate ratio are those with endosymbiotic bacteria that contribute to nitrogen metabolism.

in the levels ingested of both nutrients, coincided with the decline of tissue growth towards the end of the somatic growth phase.

Intake targets also change in evolutionary time, as animals are selected to utilise different sources of nutriment and evolve differing life history strategies. Figure 6 shows, for example, data from a comparative analysis of the optimal protein:carbohydrate ratio of 117 insect species (Simpson & Raubenheimer, 1993a). This study revealed that insects with the steepest target rail (lowest P:C ratio) were those with endosymbiotic bacteria that contribute to nitrogen metabolism.

Finally, there are cases where different species have a similar intake target, but they have evolved to reach this in very different ways. A hypothetical example is presented for a nutrient generalist and a specialist feeder in Figure 7. Below we consider a real example, in the comparison of *Schistocerca gregaria*, a polyphagous grasshopper, with *Locusta migratoria*, a grass-feeding specialist.

We stress that the example in Figure 7 describes a *nutrient generalist*, being an animal whose diet encompasses a wide area of nutrient space, as opposed to *food generalists*, which feed on a wide range of food types. This is a potentially important distinction, since food generalists sometimes might not also be nutrient

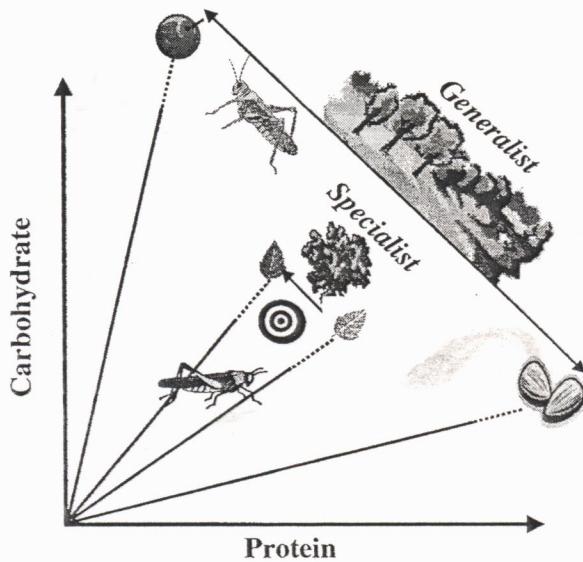


Figure 7. Hypothetical example where the intake target remains unchanged over evolutionary time, but the means of reaching it diverges between species. A nutrient generalist species might use a substantially different nutritional landscape to achieve the same nutrient intake as a specialist.

generalists. On the contrary, one reason for including a large number of food types in a diet might be to avoid any changes (e.g., seasonal or developmental) in the composition of the host(s), thus regulating nutrient intake within a limited region of nutrient space. Food specialists, by contrast, might be nutrient generalists to the extent that changes in the composition of their hosts leave them little option but to deviate from the target across wide areas of nutrient space.

Nutritional conflicts: when the intake target cannot be reached

Rules of compromise. Above, we have seen that animals are capable of achieving the intake target when they are fed nutritionally balanced foods, or are able to switch among nutritionally imbalanced, but complementary, foods (Figure 2a and c). However, animals might frequently have available neither balanced nor complementary foods, and thus be forced to ingest foods that are to some extent imbalanced. In this predicament, the animal confronts a situation of conflict among its requirements for different nutrients (Raubenheimer, 1992). As is illustrated in Figure 8a, it can satisfy its requirements for carbohydrate by feeding to point a, but in so doing will suffer a deficit of protein. At the other extreme, it could satisfy its re-

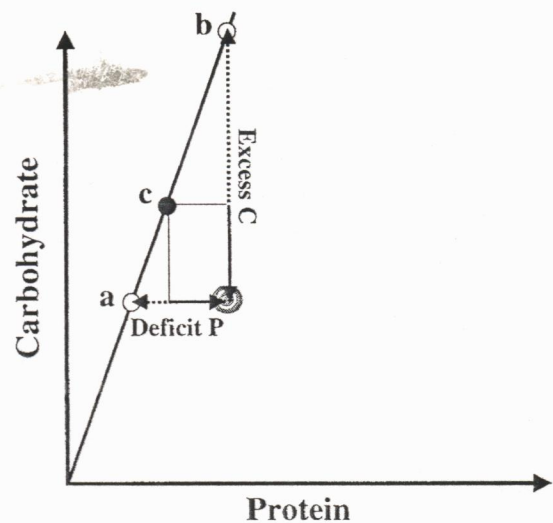
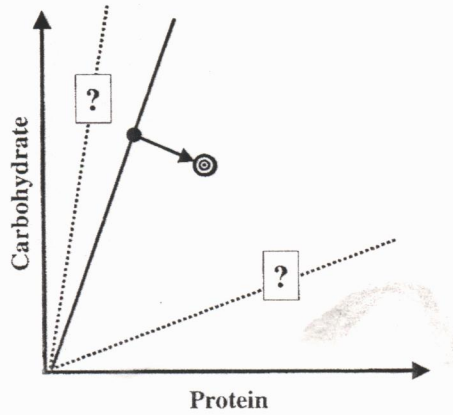


Figure 8. Nutritional compromises when feeding on imbalanced foods. An animal that feeds to point a satisfies its requirements for carbohydrate, but suffers a deficit of protein. By feeding to point b it ingests sufficient protein but an excess of carbohydrate, while at point c it experiences both an excess of carbohydrate and a deficit of protein, neither of which are as large as the extremes.

quirement for protein (point b), but only if it ingests an excess of carbohydrate. Alternatively, it could feed to an intermediate point (e.g., point c) at which it suffers both an excess of one nutrient and a deficit of the other, but neither of which are as large as the extremes. The regulated point of intake in such circumstances – the *rule of compromise* – should reflect the relative costs and benefits of over-ingesting one of the nutrients and under-ingesting the other (Raubenheimer & Simpson, 1993, 1997; Simpson & Raubenheimer, 1993a). Therefore, like intake targets, rules of compromise are an important nutritional datum, which might differ both among species and within species at different growth stages and for different combinations of nutrients.

But how to measure such rules? Having determined the position of the intake target, the rule of compromise can simply be determined by confining an animal to a single nutritionally imbalanced food and recording its intake of the relevant nutrients, as depicted in Figure 9a. This, however, provides a very limited picture, as there is no guarantee that the rule will not differ for foods that are imbalanced to a greater or lesser extent, or indeed for those that are imbalanced in the opposite direction (excess P relative to C). A fuller picture can be obtained by confining insects to a range of foods, and measuring their intake across the relevant period (Figure 9b). The array

a. Local rules



b. Global rules

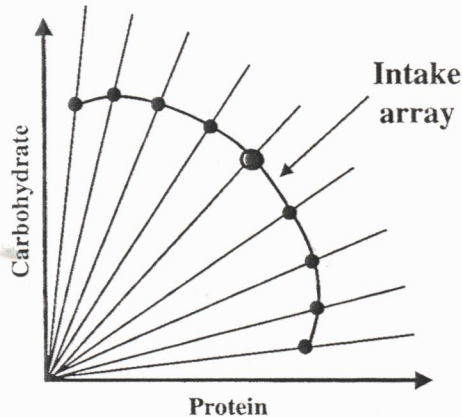


Figure 9. A single intake point provides information about the rule of compromise local to the nutritional rail (a), but says nothing about the rule on other foods. The intake array resulting from an experiment including rails representing a large area of nutrient space characterises the global rule of compromise (b).

of intake points resulting from such an experiment provides a comprehensive description of the rule of compromise for the nutrients under consideration.

Interpreting intake arrays. Among the intake arrays recorded in this manner for insects are, firstly, the response of *Locusta migratoria* to mineral salts when forced into conflict with a balanced complement of protein and carbohydrate [Figure 10; data from Trumper & Simpson (1993)]. Secondly, the intake arrays for protein and carbohydrate have been measured for the generalist locust *Schistocerca gregaria* (Figure 11a), and the grass feeder *Locusta migratoria* (Figure 11b) (Raubenheimer & Simpson, in prep.). Superimposed on the data are computer-generated

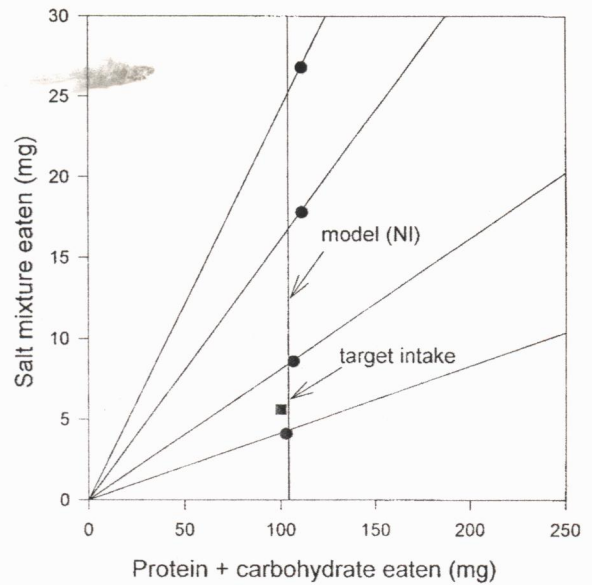


Figure 10. Intake array for 5th stadium *L. migratoria* made to trade off a mineral salt mixture against a compound axis of protein and digestible carbohydrate. The vertical line represents the prediction for the 'no interaction' rule of compromise.

models of the single rule that best fits the data in each case.

Intake arrays thus provide a visual representation of the patterns of nutrient intake when animals trade-off the over- and under-ingestion of nutritionally imbalanced foods. But how to interpret what the various rules mean? To illustrate, we will use the model data superimposed on the three intake arrays observed in locusts (Figures 10 and 11). For clarity we assume in the models a symmetrical intake target, but the interpretation would apply equally for an asymmetrical target. The model data are plotted in Figure 12.

Since intake arrays represent the rules adopted in trading off nutritional errors (excesses and deficits), they can best be interpreted by examining these errors, as illustrated in Figure 13. Plotted on the y-axis in these summary plots is the nutritional error (distance from the X and Y co-ordinates of the intake target) experienced by the animals on each nutritional rail (X-axis). The horizontal line at zero error depicts the target position (no error for either nutrient), while a negative error represents a deficit and a positive error an excess of a nutrient.

It can readily be seen in these terms (Figure 13a) what the array in Figure 12a (and Figure 10) means. These animals have regulated intake so as to satisfy without error their requirement for the combined com-

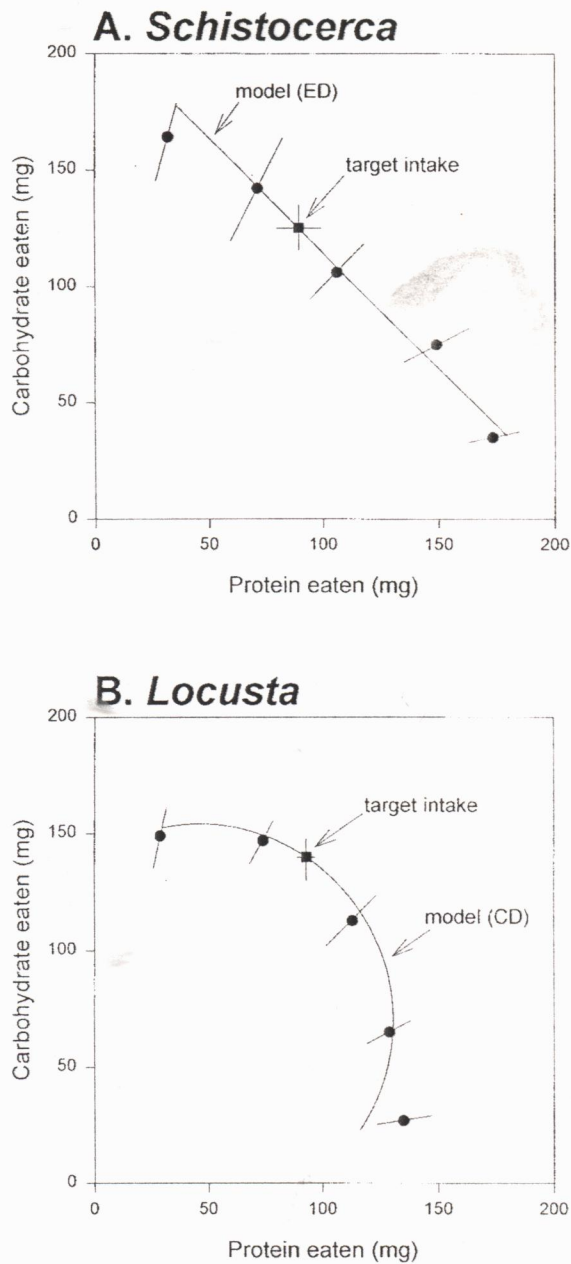


Figure 11. Protein-carbohydrate intake arrays for (A) the generalist feeder *S. gregaria* and (B) the grass feeder *L. migratoria* over the first 3 days of the 5th stadium. Filled circles represent means \pm SE of 10 insects fed one of 5 foods varying in digestible carbohydrate and protein. The solid squares represents the selected intake points of locusts allowed to switch between foods 14:28 and 28:14 (%protein:%carbohydrate). The solid lines represent the prediction for the equal distance (A) and the closest distance (B) rules of compromise.

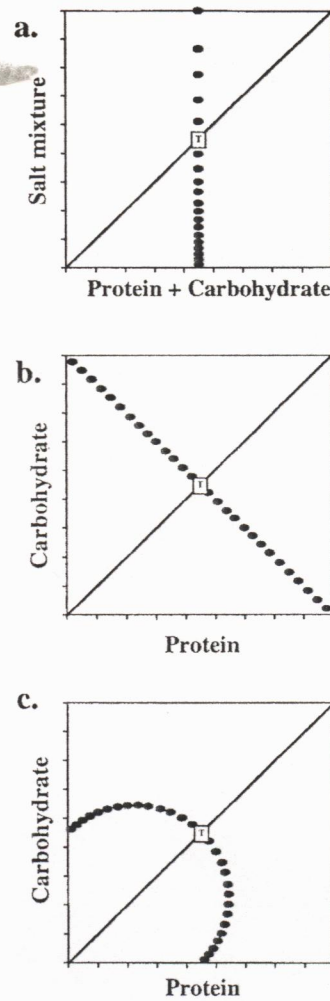


Figure 12. Model data for the no interaction (a), equal distance (b) and closest distance (c) rules of compromise.

plement of protein and carbohydrate, irrespective of the excess or shortfall of mineral salts ingested. Therefore, from the perspective of regulatory mechanisms, there has been no interaction between the two groups of nutrients, and this rule is accordingly termed the *no interaction rule* (NI).

Analysis of the equivalent plot for the intake array in Figure 12b (and Figure 11a) reveals that the rule of compromise displayed by these animals is to regulate intake such that the deficit incurred in one nutrient exactly matches the excess incurred in the other. In other words, these animals regulate to a point in nutrient space which is equidistant from the target in the two dimensions, and this rule is thus termed the *equal distance rule* (ED).

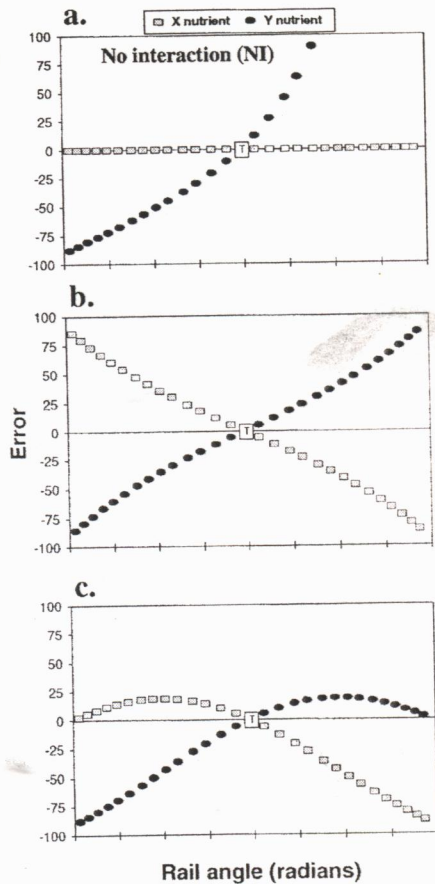


Figure 13. Plot of nutritional errors for three rules of compromise. A positive error on the y-axis represents the excess and a negative error the deficit of nutrients ingested by animals on each nutritional rail (x-axis). It is only animals on the target food (boxed T) that have no error.

Considering the error plot for the rule depicted in Figure 12c (and Figure 11b), a first striking point to note is that these animals are prepared to suffer a relatively large shortfall of the deficient nutrient (whether it be protein or carbohydrate) in order to avoid ingesting a relatively small surplus of the excessive one (Figure 13c). This result emphasises very strongly the importance of an integrative approach to nutrition, in contrast with the uni-dimensional approaches which consider the deficient nutrient to be the one that is primarily limiting (see above).

Beyond this, it is not easy to interpret in these terms what the pattern in Figure 13c means. To assist, a further summary plot is presented, which shows the relationship between rail angle and the sum of the absolute value of the two errors (protein error + carbohydrate error) incurred by animals fed each

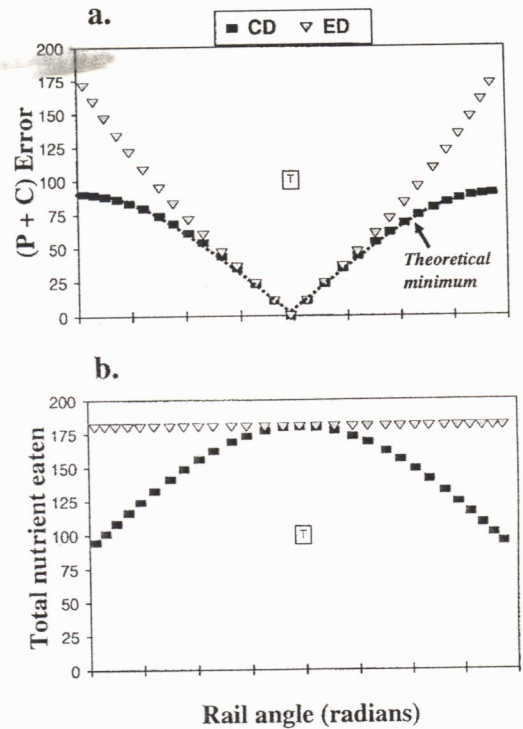


Figure 14. The sum of nutritional errors (a) and the total nutrients ingested (b) by hypothetical animals adopting the closest distance (CD) and equal distance (ED) rules of compromise. The dotted line in a represents the theoretical minimum possible for this variable.

food (Figure 14). This is, in other words, a plot of rail vs. the two-dimensional distance from the target achieved by animals fed the various foods. Figure 14a shows that this value is considerably lower for the rule in Figure 12c than for the equal distance rule. In fact, the former represents the minimum possible two-dimensional distance that can be achieved from the intake target. Therefore, animals following this rule feed to the point in nutrient space which, for a given food rail, represents the closest possible geometrical (in this case two-dimensional) distance from the intake target. Accordingly, we have termed this rule the *closest distance* (CD) rule (Raubenheimer & Simpson 1993).

This analysis provides an interesting basis for comparing the nutritional strategies in protein and carbohydrate regulation of the generalist locust *S. gregaria* (Figure 11a) with that of the grass specialist, *L. migratoria* (Figure 11b). Figure 14a suggests that the grass feeder has evolved to minimise the total deviation in nutrient space from the intake target incurred when feeding on imbalanced foods. By contrast, the generalist incurs a considerably larger error; how, then,

might they benefit from this strategy? Figure 14b suggests one potential explanation. This figure shows that the total amount of nutrient (protein + carbohydrate) eaten by the generalist is constant, and on imbalanced foods considerably greater than for the specialist. Perhaps this reflects the greater probability that a generalist will subsequently encounter an edible plant with a complementary imbalance. The ingested excess from the current food would then balance the deficit in the subsequent one, thus converting excess into useful nutrient. Overall, therefore, this comparison suggests that the grass specialist feeder *L. migratoria* has adopted a strategy of error minimisation, while the generalist *S. gregaria* is a nutrient maximiser.

Post-ingestive regulation

Our data therefore demonstrate that, where available foods permit, locusts regulate homeostatically to a point in protein-carbohydrate nutrient space, the intake target. When constrained from reaching this point, they show a clearly defined strategy of compromise which differs for the generalist feeder *S. gregaria* and the grass specialist *L. migratoria*. In this section we demonstrate a further capability in their already impressive arsenal of homeostatic responses, post-ingestive regulation.

By definition, animals that reach the intake target ingest an optimal amount and balance of nutrients, and are thus able to allocate nutrients optimally to growth. We have termed the optimal levels of nutrients that can be allocated to growth the *growth target* (Raubenheimer & Simpson, 1993). Post-ingestive regulation enables animals feeding on imbalanced foods to reach their growth target, even though they are unable to reach the intake target.

This is illustrated for *L. migratoria* in Figure 15 (data from Raubenheimer & Simpson 1993). The figure shows, firstly, the protein-carbohydrate intake array measured across the duration of the 5th stadium (in contrast with Figure 11b which shows the equivalent array measured over the first 3 days of the stadium). The arc of the closest distance array of intake points observed on the earlier measurement is still to some extent visible for insects fed the more moderate (central) foods, represented in the figure by filled circles. However, the animals on the more extreme foods have now moved outwards from the closest distance intake array. This is most pronounced in the near-vertical

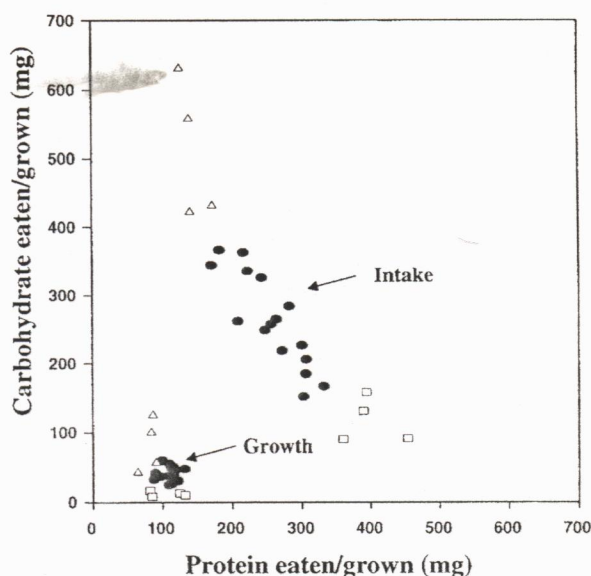


Figure 15. Protein-carbohydrate intake and growth arrays over the 5th stadium of *L. migratoria*. Filled circles represent these variables for insects fed the less extreme foods, while those fed foods with a relatively large excess of carbohydrate and proteins are represented by open triangles and squares respectively.

arm formed by animals fed foods containing excess carbohydrates (hollow triangles), but also apparent for those fed the excess protein foods (hollow squares). We will return to this change in shape of the intake array shortly.

Also shown in Figure 15 are the amounts of protein and carbohydrate allocated to growth by each group of insects represented in the intake array. This figure therefore provides a geometrical representation of a two-dimensional nutrient budget (Raubenheimer & Simpson, 1995), showing the amounts of ingested nutrient that were allocated to growth by each group of animals. The most striking result is how closely the growth points – and particularly those for animals fed the less extreme, central foods – clustered in comparison with the wide range of intake points, this cluster presumably encompassing the growth target. The extent of this regulation is shown in Figure 16, where the amount of nutrient ingested and allocated to growth are plotted separately for protein and carbohydrate against rail number. The vertical distance separating the growth and intake points for each rail represents the amount of nutrient ingested but not allocated to growth – in other words that which was eliminated from the system. The figure shows very clearly how, through regulating this latter quantity, growth was held constant in the face of fairly severe perturbations to

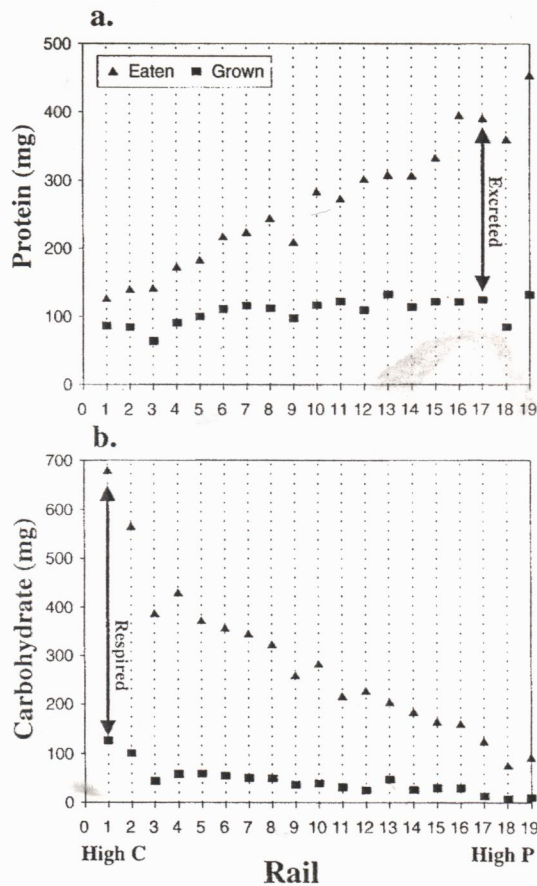


Figure 16. Growth and intake of protein and carbohydrate by *L. migratoria* fed one of 19 foods varying in the balance of these nutrients (x axis). The vertical distance between growth and intake points represents the nutrient eliminated from the animals. In the case of protein, this was via the faeces in the form of uric acid, while excess carbohydrate was respired.

nutrient intake. Experiments have shown that in these locusts excess ingested protein is de-aminated and the nitrogen excreted via the faeces as uric acid, while excess carbohydrate is metabolised through an increased metabolic rate and the carbon component eliminated as carbon dioxide (Zanotto et al., 1993, 1994, 1997).

A further interesting aspect of Figure 15, are the reasons the animals on the extreme foods broke away from the closest distance intake array. The formation of the near-vertical arm on the foods with the greatest excess of carbohydrate is due to these animals having extended the stadium duration and thus continued to feed for up to 5 days longer than those on less extreme foods (Raubenheimer & Simpson, 1993; see also Figure 17b). A noteworthy feature, is how these intake points aligned with the protein co-ordinate of

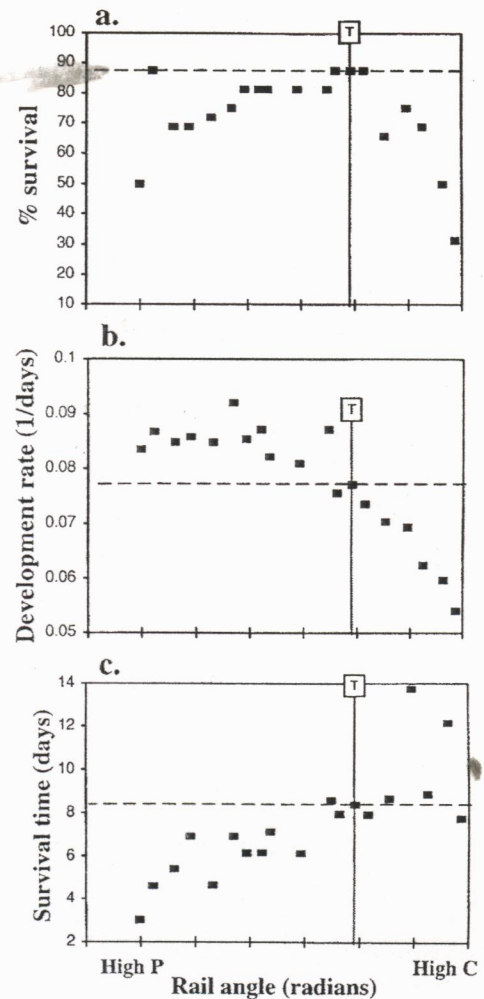


Figure 17. Performance of 5th stadium *L. migratoria* fed foods varying in protein-carbohydrate balance (x -axis). The target rail is indicated by the vertical lines.

the growth target, suggesting that the animals delayed the moult until some minimum level of protein-derived growth had been achieved. In so doing, however, they over-extended their ability to void the excess ingested of carbohydrate, resulting in them exceeding the growth target in the carbohydrate dimension. This can also clearly be seen in Figure 16b, as a breakdown in regulation of carbohydrate-derived growth.

Animals fed the foods with the greatest excess of proteins also moved outwards from the central array. Unlike those fed an excess of carbohydrate, this was not a result of extended stadium duration, since these animals moulted at a similar time or even sooner than those on the central foods (Raubenheimer & Simpson, 1993; Figure 17b). In fact, Figure 11b shows that even

after 3 days on the foods, there was already a tendency for these animals to move outwards from the closest distance intake array, demonstrating a greater ability to ingest an excess of protein than would be predicted by the closest distance rule. One possible explanation for this is that the carbon-based breakdown products of excess ingested protein could be channelled into partly offsetting the deficit of carbohydrate ingested on these foods. This would not only contribute directly to carbohydrate requirements, but would also reduce the accumulation in the organism of excess protein, thus facilitating additional intake of carbohydrate.

Performance measures

Despite the impressive ability of locusts to achieve balanced growth across a wide range of nutrient intakes, it remains a fact that when given suitable complementary foods they regulate to a specific point in nutrient space. This gives rise to the question of what are the benefits of selecting this point, rather than some other point from which they could achieve similar growth. Such functional questions are addressed in our framework using an additional axis, the *performance axis*, which maps onto nutrient space the consequences to an animal of being at a given point at a given time.

In Figure 17 is presented three performance measures for *L. migratoria*. The first plot shows that the percentage survival through the fifth stadium was low for animals fed foods containing an excess of protein, then increased up to the target rail, and once again decreased on foods containing an excess of carbohydrate. Development rate (Figure 17b), by contrast, was highest for animals fed the excess-protein foods, then dropped towards and beyond the target rail. This demonstrates that these locusts could in fact achieve a higher development rate than they do under ad-libitum conditions, by selecting an intake point closer to the protein axis – why don't they?

While a comprehensive answer would require a detailed analysis of various performance measures, one possible reason is suggested in Figure 17c. This shows the survival time of locusts brought through the 5th (final) larval stadium on one of the foods then deprived of food as adults – it is, in other words, a measure of resistance to starvation. The figure shows that animals fed the high protein foods succumbed to starvation several days earlier than those fed foods containing relatively higher levels of carbohydrate. Therefore, increasing development rate by selecting

diets with higher protein content would make these locusts vulnerable to food shortages. Quite possibly, the selected intake point represents a prioritisation of survival through the fifth stadium, together with a balanced trade-off between development rate and resistance of adults to starvation.

Nutritional dimensionality

Our data have demonstrated that for locusts, a large proportion of the variance in nutritional regulation and performance can be explained through the interaction of two nutrient groups, proteins and carbohydrates. Many foods are, of course, more heterogeneous than this, as are the nutrient requirements of animals. This gives rise to the important issues of how to select the number of dimensions to be included in a model, and how to expand the geometrical approach to consider more than two dimensions.

The number of relevant dimensions depends, obviously, on the biological details of the system in question, but also on the research approach being taken. At the one extreme are studies of insect behaviour on, for instance, whole plants in the wild, where the researcher has little or no control over the chemical composition of the foods. In such cases, a range of chemical variables might be measured and attempts made to map the animals' responses onto underlying nutritional patterns. Here, determining the number of relevant dimensions would be a standard statistical issue of identifying the nutrients that explain a good proportion of the variance in the animals' nutritional responses, while not over-saturating the model with redundant terms (Wright, 1998). At the other extreme are laboratory studies using synthetic foods, where the number of relevant dimensions can to some extent be determined by the experimenter, as discussed further below. Somewhere between the two is the use of genetically manipulated plants, where key nutrient groups are controlled within a complex plant physiological milieu (Wright, 1998).

Whether in a field-type study or a laboratory experiment using synthetic foods, the geometrical approach can readily be extended to analyse data of dimensionality exceeding two. One approach would be to use three-dimensional graphical representations. While this might be heuristically useful as a graphical illustration of increased nutritional dimensionality that is within the conceptual grasp of non-mathematicians, in practice it has limitations. Firstly, it does not readily

extend to more than three dimensions, and secondly it is in our experience frequently difficult to interpret three-dimensional scatter plots visually. A second approach, and one that we have found useful in the study of three-dimensional systems (Simpson & Raubenheimer, 1997), is to reduce a multi-dimensional representation to a series of two-dimensional cross-sections. There are several advantages to this approach, including its simplicity and the fact that it can cope with any number of dimensions. However, since the number of cross-sections increases geometrically with the number of dimensions, there are advantages to restricting the number of dimensions included in a model.

One means of reducing the number of dimensions is to use *compound axes*, which represent more than one nutrient (Simpson & Raubenheimer, 1993, 1995). There are several contexts in which this approach might be applied. For example, including all amino acids separately in a model will yield a cumbersome explosion of parameters, so it may be preferable to represent them jointly on a single, compound, protein axis. This sort of coarse-graining might be more than a methodological convenience, but potentially a fair reflection of reality, since insects are themselves incapable of regulating separately the intake of all amino acids (Simpson et al., 1990; Simpson & Simpson, 1992). A second reason for compounding axes is where two or more nutrient groups are functionally interchangeable. For instance, many vertebrates channel both ingested carbohydrates and lipids into energy metabolism, and these nutrients can therefore appear on a single axis representing energy. Likewise, amino acids might all constitute sources of nitrogen, and may therefore be represented as a single axis. A third application of compound axes is where two or more nutrients are present in a food in approximately balanced proportions. Since in this case there is no conflict among the nutrients concerned – once the animal has satisfied its requirements for one, it has done so for all – they can reasonably be considered a single resource and tested jointly in pairwise models against other nutrients. Trumper & Simpson (1993) used this approach to investigate the regulation of salt intake in 5th stadium *Locusta migratoria* Figure 10.

This latter experiment, and several unpublished studies involving vitamins and other micro-constituents of the synthetic foods we have used in our research into locust nutrition, explains our focus on a protein-carbohydrate two-dimensional model. Jointly, the evidence suggests that among the dry diet compo-

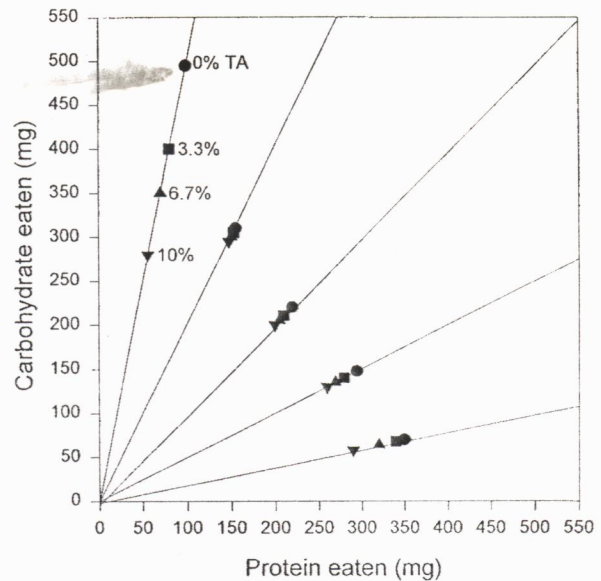


Figure 18. Protein-carbohydrate intake arrays across the 5th stadium for *L. migratoria* fed foods containing one of four levels of tannic acid.

nents protein and carbohydrate explain a substantial proportion of the variance in nutritional regulation in these insects. While this explains our interest in protein and carbohydrate, it does not justify our omission of the micronutrients from the models, an issue which does require explanation since it is known that many are indispensable components of an insect's diet (Dadd, 1960). We have dealt with this by ensuring, firstly, that all micronutrients are present in the foods at levels that support good growth and development. Secondly, the use of non-nutritional crystalline cellulose in the foods enables any manipulations of the levels of macronutrients to be compensated, such that the relative concentrations of all other components remain unchanged (Simpson & Abisgold, 1985; Raubenheimer & Simpson, 1993).

There remains, however, an important macronutrient not represented in any of the models presented in this review: water (but see Raubenheimer & Gäde, 1993). The way we have dealt with this in the work presented above, illustrates a further approach to reducing dimensionality, namely by providing one or more nutrients separately from the rest. This allows the animals to regulate the separate nutrient(s) orthogonally, such that there is no conflict between them and the other nutrients.

Allelochemicals

Finally, the geometrical approach is not restricted to nutrients, but can readily incorporate non-nutritional food components such as allelochemicals. An example of the influence of allelochemicals on intake arrays is taken from S. J. Simpson & D. Raubenheimer (unpubl.). This study showed that tannic acid reduced nutrient intake by *L. migratoria*, but only on foods containing a large excess of carbohydrate relative to protein (Figure 18). This contextual effect was echoed in performance (mortality). Interestingly, while there was little effect of tannic acid on consumption by insects fed excess-protein foods, tannic acid did increase mortality on these foods, suggesting a post-ingestive toxic effect. These data therefore emphasise a point that is central to our motives in developing the geometrical approach: The influence of a given factor (in this case an allelochemical) on a biological system can be heavily dependent on the context in which it occurs (in this case nutritional balance), and an understanding of the system requires systematic study of both the factor and the context. It requires, in other words, an integrative approach.

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