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A multi-level analysis of feeding behaviour: the geometry of nutritional decisions

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SUMMARY

We have presented and tested in experiments with insects a new framework which integrates functional, mechanistic, ontogenetic and comparative aspects of nutrition. The framework firstly identifies local optima (nutritional, intake and growth 'targets') within a multi-dimensional nutritional space, where each functionally relevant nutrient forms a single dimension. The positions of these targets are located experimentally through studies of feeding behaviour and physiology and their functional significance is tested using independent performance criteria. Functional rules employed by animals to either reach these targets or, if that is not possible, find a point of best compromise, are then investigated by reference to the geometry of arrays of intake and growth across a range of foods. Changes in the position of the targets with ontogeny are considered, as are the nature of underlying homeostatic mechanisms. We also show how comparative analyses of a range of species can be used to investigate the influence of non-nutritional, ecological factors on the location of the targets.

1. INTRODUCTION

The fact and functional significance of the regulation of food intake are axiomatic: 'Food is essential to life and failure to regulate intake at least over the long term does not favour a large posterity' (Staddon 1983). Less certain are (i) the local functional optima, and (ii) the behavioural and physiological mechanisms whereby these optima are achieved. That is, what is it that feeding behaviour has evolved to achieve, and how is the discrepancy minimized between this and what is actually achieved? These are, respectively, what Tinbergen (1963) has termed functional and mechanistic explanations of animal behaviour.

Both questions are currently the focus of considerable interest in Optimal Foraging Theory (henceforth, OFT). There, the approach has been to construct, and test empirically, optimality models of behaviour which maximize, subject to specified constraints, some 'currency' (Schoener 1971) assumed to be commensurate with evolutionary fitness. Qualitative and quantitative hypotheses are made about the behavioural response of the animal to the environment, and experimentation and observation are used to improve, in a step-wise manner, the correspondence between the model and reality (see Kacelnik & Cuthill 1987). The aim is to understand animal behaviour, and not merely to forecast it (Hobbs 1989): i.e. to model rather than simulate (*sensu* Johnson-Laird 1983). Despite the widely acclaimed success of this approach (Krebs & Kacelnik 1991; Parker & Maynard Smith 1990; Schoener 1987), there have been strong statements of dissent (Lewon-

tin 1978; Gray 1987; Ollason 1987; Pierce & Ollason 1987).

OFT is therefore a 'top-down' approach, in which functional expectations are used as a starting point to understand the evolutionary origins and mechanistic basis of behaviour. Another approach is to start with a description of the nature and dynamics of underlying mechanisms, and from there work by inference towards evolutionary and functional considerations. The premise underlying such inference is that mechanisms represent the outcome of natural selection, and their design features should therefore shed light on past selective pressures (i.e. functional explanations for behaviour).

Here we introduce a 'bottom-up' framework for investigating the nutrition of animals, and in principle also plants. This, a geometrical approach, investigates the dynamical interactions among mechanisms regulating the intake of specific nutrient groups. Similar ideas were anticipated in the excellent theoretical work of McFarland & Sibly (1972, 1975). Our aims are, firstly, to describe the framework and to investigate and test it using the African migratory locust, *Locusta migratoria*. Secondly, we consider its use as a basis for integrating functional, ontogenetic, comparative (evolutionary) and mechanistic approaches to the study of feeding and nutrition, again using insects. We do not view 'top-down' and 'bottom-up' approaches as alternatives, but rather as complementary, interactive means of developing a multi-level understanding of animal behaviour. This was stated by Tinbergen (1963), and latterly reiterated by Dawkins (1989), to be a major aim of ethology.

2. THE GEOMETRIC FRAMEWORK

(a) *Targets, dimensions and currencies*

Animals require, simultaneously, a wide range of nutrients (here used to include proteins, carbohydrates, fats, micro-nutrients and water) to provide for growth, reproduction and other metabolic activity. At any one time, each nutrient is required by the animal's tissues at what we will assume for explanatory purposes to be a specific, optimal level. The position of this optimum is dependent on, among other things, the animal's stage of development and current environmental circumstances. The ideal nutritional state, or that in which the requirements for all nutrients are, simultaneously, optimally satisfied, can be viewed as a multidimensional 'nutritional target', with as many dimensions as there are functionally relevant (fitness-affecting) nutrients (Raubenheimer & Simpson 1993). To the extent that nutrient requirements vary with time, the target is a moving one.

Foraging behaviour is the first step in a suite of adaptations which have the shared function of minimizing the discrepancy between the nutritional target and an animal's current nutritional state. Food is selected from the environment, then passed via ingestion down the alimentary canal to the mechanisms responsible for various levels of chemical processing. Each stage in this process engenders structural and energetic costs thus diminishing the net utility of the food, ultimately translating into increased food requirements; hence an increase in feeding time and any associated ecological costs. It may therefore be expected that feeding behaviour will be calibrated through natural selection to provide the post-ingestive machinery with each nutrient at a level that maximizes its net contribution to fitness. This level, aimed at through feeding behaviour, is referred to in our framework as the 'intake target' (Raubenheimer & Simpson 1993).

A third functional optimum is the 'growth target'. This is the optimal level of nutrients that can be incorporated into growth of the animal's tissues, including storage organs. The growth target is thus a component of the nutritional target, and differs from it by the nutrients required by the animal's other metabolic processes. The nutritional target is achieved only when neither growth nor other metabolic requirements are compromised.

Individual components (co-ordinates) of the intake, growth and nutritional targets can, theoretically at least, be measured on a one-dimensional scale. OFT has conventionally considered nutrition from the viewpoint of such a single scale, usually energy intake or some variant thereof. Other factors, such as individual nutrients, have been considered as constraints on the major currency hypothesized (Pulliam 1975; Belovsky 1990). A uni-dimensional consideration of energy intake may approximate reality for animals which have ceased growing and thus feed mainly to meet energetic demands, or where animals feed on foods which are relatively invariant in nutrient content (e.g. carnivores and granivores). In the latter

case, behaviour that maximizes energy intake will also maximize intake of all other nutrients in the food.

For growing animals, which require structural nutrients in addition to energy, and animals that feed on nutritionally heterogeneous foods (e.g. phytophages), it is more appropriate to investigate the intake, growth and nutritional targets as points in a multidimensional 'nutritional space'. In this case, the application of optimality models becomes considerably more complex. For example, it is no longer obvious which measurable dependant variable should be maximized. The currency likely to correlate best with evolutionary fitness is a composite variable, representing several simultaneous and dynamic physiological requirements. Optimal behaviour may be that which maximizes no single component of these requirements (e.g. energy intake), but attains some point of balance among them. This point of balance is the nutritional target. The position of this target will be subject to natural selection and cannot be known *a priori*, but is a matter for empirical determination.

(b) *Nutritional rails*

(i) *Feeding without a choice of foods*

There are two ways an animal can regulate feeding so as to approach the intake target: through regulating the amount of an individual food eaten, or through selecting among alternative foods. This distinction is used mainly as an explanatory convenience, as any act of feeding involves elements of both food selection and regulation of intake (Simpson & Raubenheimer 1993). We begin with the simpler case, where only one food is available, and later consider food choice.

For a single food item which contains a fixed proportion of nutritional components, intake of any one nutrient is linearly proportional to intake of all the others. An animal eating a single food type can therefore be viewed as being confined to a 'rail' in multidimensional nutrient space. The intake target is only achievable if it lies on the rail defined by the balance of nutrients in the food (i.e. if the food is nutritionally balanced with respect to the animal's current requirements).

Figure 1*a* illustrates this for a simple system, involving only two functionally relevant nutrients which are required (arbitrarily) and present in the food in 1:1 proportions. Here the animal behaves optimally by feeding until its requirements for both nutrients are met (i.e. until it reaches the target). In the short-term (e.g. following a single meal or foraging bout) the current position (denoted by X) recedes with time in the direction of the pre-feeding state and, beyond some threshold, feeding once again begins. Whether the current position moves down the rail or via some other trajectory depends on the relative rates of absorption and egestion of the nutrients involved.

Consider, however, a food that is nutritionally imbalanced, containing a 1:2 proportion of nutrients A:B (figure 1*b*). Here the animal is restricted to the 1:2 rail, and is thus unable to reach the intake target which lies on the 1:1 rail. Where no alternative food is

available, the animal has three basic options as illustrated in the figure: it can (i) feed until the target for nutrient A is reached then stop, suffering a shortfall with respect to nutrient B; (ii) feed until the target for nutrient B is reached, in which case it ingests an excess of nutrient A; or (iii) feed to some point intermediate between these extremes, experiencing simultaneously a shortfall with respect to B and an excess with respect to A.

The key point is that when foods are imbalanced with respect to two or more nutrients, conflicts may arise between the mechanisms regulating intake of the nutrients concerned (see Raubenheimer (1992) for a more detailed discussion). Which of behaviours (i), (ii) or (iii) is performed, depends on the 'decision rule' (cf. Pyke 1978) that is employed to resolve these conflicts. In so far as decision rules are considered to have evolved through natural selection, they represent points of best compromise (functionally optimal compromise). Our geometric framework aims to identify the relevant nutrients, locate the nutritional, growth and intake targets (functional optima) for these, and investigate the decision rule for resolving conflicts in the intake of these nutrients.

(ii) *Choosing among foods*

There are two levels at which an animal may benefit from the opportunity to choose among different foods. First, where there is the opportunity to select either a nutritionally balanced or an imbalanced food (e.g. the 1:1 versus 1:2 foods in figure 1*b*), the animal should only eat the former. Such considerations may play a selective role in determining which items, from among the numerous nutrient-containing substances in its ecological environment, an animal responds to as potential food. The parameters are fixed within an individual, but may change across generations.

Second, by choosing appropriately from among two or more foods, an animal may reach the nutritional target (select a balanced diet) even where none of the foods is itself nutritionally balanced. This is a finer-grained problem of nutritional homeostasis, which operates at the behavioural level. Consider, for example, figure 1*c*. Here the animal has available a 1:2 food (as in figure 1*b*) and another imbalanced food, containing two parts of nutrient A to one part of nutrient B. This animal is no longer confined to a single rail, but is free to move in two trajectories, each parallel to one of the rails. By eating various combinations of the two foods, the animal can move to any point in the plane bounded by the two rails, but cannot reach any point outside of this plane.

The sequence of behaviours whereby the animal approaches the intake target is non-arbitrary. To take an extreme example, an animal may compromise its fitness by eating exclusively from one imbalanced food for say two days then eating the other imbalanced food for the following two days, even though it may reach the intake target for four days in this way. At the other extreme, if the animal were to switch between foods too frequently, it may never consummate a feeding event. Such behavioural indecisiveness

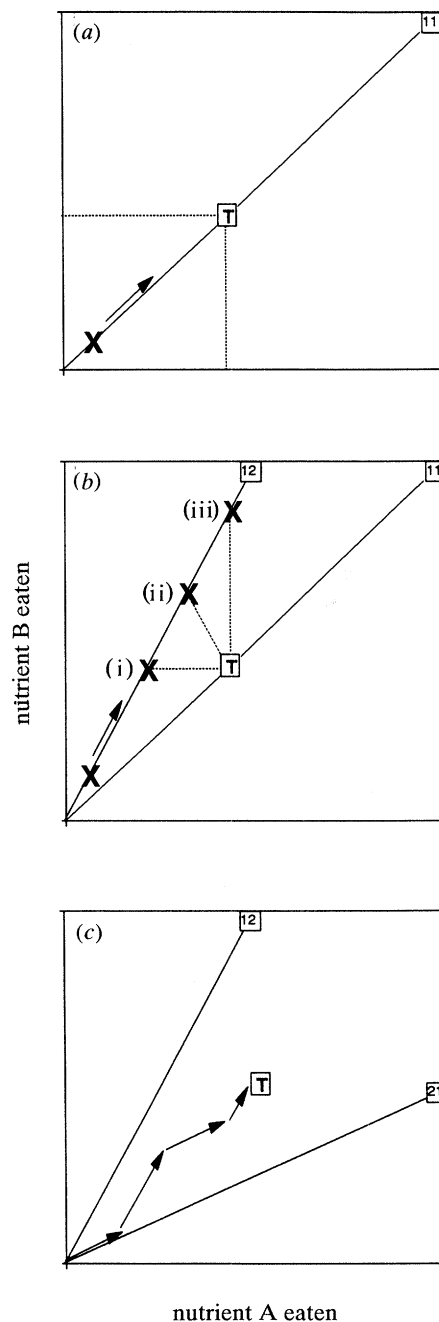


Figure 1. Nutritional planes for intake of two functionally relevant nutrients, A and B. The intake target (T) lies on the rail bearing foods containing a 1:1 ratio of nutrients A and B. The top panel (a) shows how an animal on a food containing the optimal ratio of A and B can reach the intake target by moving along the rail from its current position (X). In (b) an animal is given a food which contains 1:2 A:B and is thus unable to reach the target. Three alternative strategies are shown: (i) the animal could move along the rail until it has eaten the target co-ordinate for B, and so suffer a shortfall of A; (ii) it could eat until the target co-ordinate for A is reached, and so ingest more than the target level of B; or (iii) it could move to a point intermediate between these latter two. The bottom panel (c) shows how an animal given a choice of foods 1:2 and 2:1 is able to move freely within the space bounded by these rails, and so reach the target.

has been referred to by McFarland (1971) as 'dithering'. The optimal behaviour may be to change foods on subsequent meals, or even to switch within meals. The importance of behavioural sequences in such instances is discussed more formally by Sibly & McFarland (1976).

(c) *Examining decision rules*

Figure 1*b* depicts a hypothetical situation in which an animal has available only a food which is nutritionally imbalanced with respect to two functionally relevant nutrients. The intake target can thus not be reached, and there is a conflict between ingesting an excess of one or a deficit of the other nutrient. The same applies to choice situations in which the intake target lies outside of the plane bounded by the choice rails. In this case the animal should, optimally, feed only from the rail closest to the target, yielding a situation equivalent to the no-choice situation. To determine which decision rule is being adopted in either case, the location on the plane of two points must be known: the position of the intake target (i.e. the functional optimum) and the 'behavioural end point' (the point to which the animal actually feeds).

(i) *Behavioural end points*

Behavioural end points can be determined simply by measuring the amounts ingested of the two (or more) functionally relevant nutrients over a given time period. The duration of this period is determined by the investigator's interests, and can range from a single meal or foraging bout to a specified developmental stage (e.g. an insect stadium), or even the animal's lifetime. The crucial point is that the intake target must be estimated (as described below) over the same period.

The position of a single end point in relation to the intake target (e.g. figure 1*b*) provides information about the decision rules underlying food intake under only a single circumstance: in this case, where nutrient B is present at twice the optimal concentration relative to nutrient A. This provides a limited view of the animal's behaviour, with no indication of the dynamics involved. To obtain a more general view, it is necessary to determine whether the single-point rule is commutative to foods containing the two nutrients in a range of proportions.

Decision rules should therefore be determined experimentally with respect to a range of nutritional rails, each representing a food containing different proportions of the nutrients involved. Under such circumstances, the decision rule is expressed as the shape of an array of behavioural end points in relation to the intake target, each point depicting the amounts of the two nutrients eaten by a different group of experimental animals. The shape of the array may also, in some instances, provide an indication of the position of the intake target, as will be further discussed below. Some hypothetical arrays, representing extremes in a continuum of possible decision rules, are illustrated in figure 2.

(ii) *Determining the position of the intake target*

Locating the position of the intake target on the nutritional plane provides a means to interpret the arrays of behavioural end points in terms of best compromise decision rules. Below we discuss several means of estimating the position of this target, some or all of which can be used depending on the available information. Where independent estimates concur, this provides a strong form of inference. On the other hand, discrepancies between, for example, functional and mechanistic estimates may give rise to interesting questions for further research.

Performance criteria

Bearing in mind that the intake target is defined as the point on the nutritional plane representing the amounts of nutrients that should be eaten to maximize fitness (i.e. is functionally defined), measurement of performance criteria is, in theory at least, the most direct method of estimating the location of this point. On this basis, the intake target could be visualized as a maximum value on a third axis (the 'performance axis') for, ultimately, inclusive fitness. Two problems exist, however. First, it is usually not feasible to obtain a direct measure of inclusive fitness, so one or more other performance criteria assumed to correlate with fitness must be used. Individual reproductive performance, developmental time, and survival are all possibilities. Where more than one of these is measured, the assumption that they represent inclusive fitness is strengthened when there are appropriate covariances among these variables. Second, assuming that a single optimal point exists, it may not be possible to obtain a performance variable with sufficient resolution to distinguish this point. It may, none the less, be possible to distinguish a plateau (or in the case of developmental time, a trough) and on this basis delineate an area on the plane likely to contain the target. For example, rails on which there are low survival rates, or those that do not support growth, can be eliminated. Such regions are likely to occur as two sets of adjacent rails on the plane, separated by an area enclosing the intake target. The latter is referred to in what follows as a 'performance plateau'.

The choice of performance variables is important. They should, in general, conform to two criteria: (i) there must be strong grounds for believing that they correlate (positively or negatively) with inclusive fitness, and (ii) they should conform with *a priori* quantitative expectations such as maxima or minima. These two points can be illustrated using the example of animal mass. With reference to the first point, it is a common assumption that animals which maximize their mass also maximize their fitness. If this were true, body mass would form a suitable variable for the performance axis. This may not, however, be true. Mass increment is a crude measure of the assimilation into growth of a range of nutrient groups, the major ones being water and those derived from carbohydrate (including lipids) and protein. For example, the common practice of drying insect carcasses before calculating gravimetric budgets is an acknowledgment that at least one of these components is poten-

tially confounding. As discussed by Raubenheimer (1992), it is equally possible that an excess of either protein or carbohydrate-derived growth results in a mass increment that correlates negatively with fitness (McNamara & Houston 1990).

It might therefore be that it is not total mass increment that correlates with fitness, but the ratio of major components assimilated into growth. Where this is the case, growth is not suitable as a performance criterion, as there is no *a priori* basis on which to form quantitative expectations as to which ratio values represent higher fitness than others (point (ii) above). In order that growth be used as a means to estimate the location of the nutritional and intake targets, it is necessary first to determine which ratio of growth components is optimal (i.e. to locate the growth target). This may be inferred from the expression of the mechanisms regulating growth derived from the different nutrient groups, as described below.

Evidence from mechanisms

In general terms, the use of performance criteria as described above identifies the limits to the mechanisms involved in nutritional homeostasis i.e. the zones beyond which failure to compensate fully for nutritional imbalance is apparent in the selected performance criteria. Where this method indicates a peak on the plane, corroboration by independent means will increase the strength of inference. Where there is a plateau on the chosen performance axis, additional means are required to restrict further the region likely to contain, or preferably actually to locate, the intake target.

This may be done by measuring directly the expression of the behavioural and physiological mechanisms involved in nutritional compensation. In so far as these are considered to be the products of natural selection, it can be assumed that they will tend towards achieving functional optima thus indicating the position of the intake target. By analogy, consider that a researcher wishes to determine the optimal temperature at which to house a species of mammal about which very little is known. A behavioural approach would be to allow the animal to select an environment from among several which differed only in temperature. A physiological approach would be to have several experimental groups, each of which is kept in an environment differing in temperature from the others. The thermoregulatory mechanisms would ensure that the range of body temperatures was smaller than the range of environmental temperatures, i.e. there would be homeostatic convergence on a point (or region) within the range of environmental temperatures. It would be surprising indeed if animals constrained in a temperature outside of the selected (or regulated) range fared better by some reasonable performance criterion than those within the range. Similarly, in the case of growth, it may be expected that the outputs (growth ratios) of the homeostatic systems involved would be concentrated relative to the inputs (the food nutrient ratios) and the nature of this concentration provides information about the 'design criteria' (functional considerations) of the mechanisms

(see Rosen (1970), Hubbell (1971), McFarland (1971), Caswell *et al.* (1972) and Calow (1976) for general discussion of this systems approach). In this way, behaviour and physiology may be used to provide independent estimates of the position of the intake target. As will be clarified below, both methods benefit from a prior analysis using performance criteria.

Estimates from behaviour are achieved by providing the experimental animals with a choice of suitable foods, thus allowing them to indicate the preferred position in nutritional space. The decision as to which foods are suitable for inclusion in such a test is an important one. Consider, for example, the situation illustrated in figure 1c in which two functionally relevant nutrients are involved and each animal has a choice of two foods with different proportions of these nutrients. The more different these foods are in terms of the ratio of the two nutrients, the wider apart are the representative rails and therefore the more likely that the area available to the animals contains the intake target. In the extreme, each food may contain 0% of one nutrient so that the rails would converge with the axes and the entire nutritional plane would be accessible. On the other hand, as the choice rails diverge widely from the rail containing the true target, so the foods become more unnatural and the likelihood increases that the animals will respond pathologically. Thus, a food lacking in phagostimulatory carbohydrates might not be eaten at all, giving the false impression that this class of nutrient is not functionally relevant. Also, artificial foods that differ widely in nutrient content may differ in other, confounding, aspects such as texture.

Suitable foods to be included in such an experiment may be identified using performance criteria as outlined above. A plateau on the third axis would delineate a region known to contain the intake target, at the same time excluding rails with a high probability of eliciting a pathological response. The foods of choice would be those representing the rails delineating the plateau.

It is important to stress that the outcome of such a choice experiment (i.e. the observed behavioural end point) is only a satisfactory indication of the intake target if it reflects the homeostatic mechanisms regulating intake of the nutrients under investigation. For example, if the position of the intake target in figure 1c were not known, the observed behavioural end point could be explained equally as the outcome of three possible processes: (i) through regulation for a 1:1 proportion of nutrients A and B (indicating the true position of the intake target); (ii) through regulation for some third nutrient, not accounted for by the axes involved; (iii) through random feeding on the two rails resulting, on average, in half of the intake coming from one and half from the other food.

To distinguish the first from the other possibilities, the animals must be made to 'defend' the putative target. This is done by forcing two or more experimental groups of animals to behave differently to achieve the same outcome (behavioural end point) or, conversely, to achieve a different end point by behav-

ing similarly. Thus, a group of animals allowed to choose between foods containing nutrients A and B in 1:2 and 2:1 proportions would take half their intake from one and half from the other rail to reach an intake target on the 1:1 rail (as in figure 1c). By contrast, a second group given foods 1:2 and 4:2 would have to eat relatively more of the former to achieve the same behavioural end point; if they ate the same amount of both foods, the behavioural end point would be midway between the 1:1 and the 2:1 rails.

Defence of a target provides evidence for the existence and position of the target for the nutrients involved. By contrast, the failure to defend an intake target is not as informative a result. It could indicate: (i) that the nutrient(s) under consideration are not functionally important (i.e. they do not contribute a co-ordinate to the target) so no homeostatic mechanisms have evolved to regulate their intake; (ii) that the nutrients are important, but in the natural habitat of the animals suitable levels are obtained through correlation with regulation for other nutrients; or (iii) that the mechanisms do exist but are not expressed under the parameters of the experiment. This may be the case where nutrients are regulated through general means (Rozin 1976; Simpson & Raubenheimer 1993), such as learning, which may not be expressed over the timecourse of the experiment, or for other reasons of experimental design (e.g. where artificial foods are used, there may be no suitable conditioned stimuli). Another example may be where regulation does take place but only when bodily stores are depleted to an extent not realized in the experiment.

In addition to behaviour, estimates of the intake target may be derived directly from physiology. Nutrients ingested must meet the animals requirements for growth and other metabolism, which together constitute the nutritional target. To the extent that there is incomplete efficiency at the various stages of post-ingestive processing, the animal must ingest more of each nutrient to achieve the nutritional target. Stated formally, the relationships among these components for a particular nutritional axis are as follows.

Because

$$N = G + M, \quad (1)$$

and

$$I = N \times U^{-1}, \quad (2)$$

therefore

$$I = (G + M) \times U^{-1}, \quad (3)$$

where N is the co-ordinate of the nutritional target for that nutrient, G is the co-ordinate for the growth target, M is the nutrient's contribution to other metabolism, U is the efficiency with which the nutrient is utilized and I is the co-ordinate of the intake target.

From equation (3) it follows that the intake target can be estimated from measurements of the utilization

efficiencies and the contribution to growth and to energy and other requirements of each nutrient. The question is, which foods should be used to obtain the required parameters of growth, other metabolism and utilization efficiencies? As the intake target is the ideal intake point, it follows that this method is valid only to the extent that the variables on the right hand side of equation (3) have parameter values representative of animals fed the diet on the optimal rail (i.e. that which passes through the intake target). Clearly, to avoid circularity we need independent criteria to determine which rail yields the optimal parameters. As a first step, the range of eligible rails can be restricted using performance criteria as described above. Mean growth and metabolism values for animals on the performance plateau may be used, but on its own this yields little more than a recovery of the information used to identify the plateau.

Independent information can be obtained from the expression of physiological mechanisms. Consider a single two-axis plot containing the rails identified as eligible using performance criteria (i.e. the performance plateau), the behavioural end points for animals fed those foods and, plotted on the same axes, the amounts of growth derived from the two nutrients by each experimental group of animals ('growth points'). If the growth points span a similar range of rails as the behavioural end points, this indicates that the amount of growth in the two dimensions is a passive consequence of nutrient intake and not regulated independently. If, by contrast, the growth points concentrate to some extent, and it is assumed that this concentration is the expression of mechanisms evolved to regulate the incorporation of the nutrients concerned into growth, then the direction of this concentration provides a basis for an improved estimate of the position of the intake target. In other words, one term on the right hand side of equation (3) has been improved in terms of optimality estimates, and to this extent so has the estimate of the intake target.

On the assumption that natural selection favours metabolic efficiency, measures of energy expenditure can further improve estimates of the intake target. Consider, for example, that the mechanisms regulating growth were perfect, and all the animals in the above example reached the growth target. To derive an estimate of the intake target, this point must be corrected for the remaining terms on the right hand side of equation (3), other metabolic expenditure and utilization efficiency. Given that all the animals achieved the same growth point, it seems reasonable to conclude that those that achieved this with minimal energy expenditure performed more optimally than the others. The concentration of growth points can therefore be corrected using the minimum measured energy expenditure to provide an estimate of the nutritional target (equation 1).

The nutritional target differs from the intake target by the coefficient for utilization efficiency (equation 2). Once the levels for growth and other metabolism have been fixed (i.e. the optimum located), utilization efficiency is a deterministic inverse function of nutrient intake. If it still remains to decide which from among

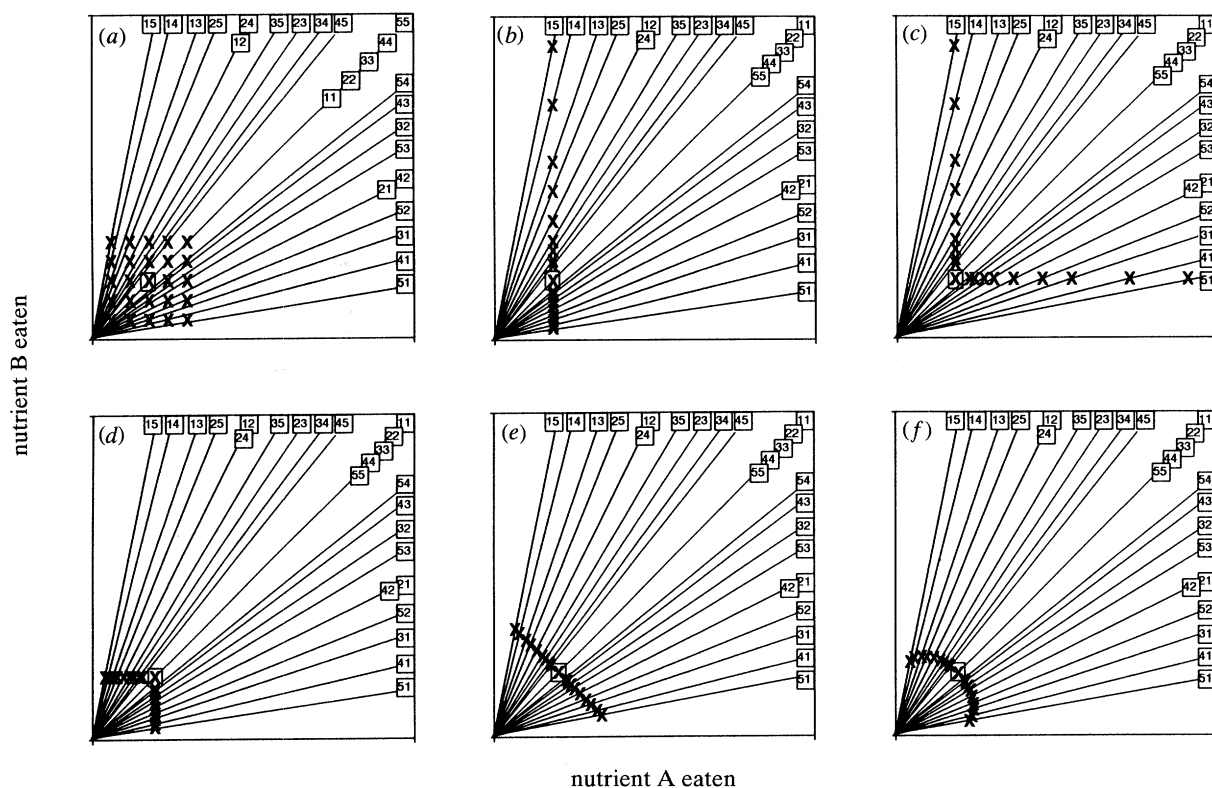


Figure 2. Nutritional planes from an hypothetical study in which animals were fed one of 25 foods, containing one of five levels each of two nutrients A and B. Amount eaten of B is plotted on the y -axis against amount of A eaten on the x -axis, with the ratio of A and B in each food being shown in boxes at the end of each nutritional rail. The first digit of this number gives the level (1–5) of nutrient A present in the food and the second digit represents the level of B. Each of the panels indicates the array arising from a particular functional feeding rule. The square on each panel is the intake target. (a) Rule 1: eat the same volume of food, irrespective of how much A and B it contains. (b) Rule 2: eat until the level of the intake target for A is ingested, irrespective of how much B is consumed. (c) Rule 3: eat until at least the levels of the intake target are reached for both A and B, irrespective of whether more than the target level for one is eaten to reach the target level for the other. (d) Rule 4: eat until the level of the intake target is reached for either A or B. Stop eating once the target level has been reached for one of the nutrients. (e) Rule 5: eat until the sum of A and B ingested equals the sum at the intake target. (f) Rule 6: eat until the point on the rail is reached which is geometrically closest to the intake target ('closest distance optimization').

two or more experimental groups that supported optimal growth and energy expenditure bears the intake target, that which allows maximal utilization efficiency (hence minimal intake and costs associated with feeding) is the more plausible.

Shape of the array

The shape of the array of behavioural end points can provide corroborative evidence for the position of the intake target. This method of inference is based on the premise that any given shape of array can be related more parsimoniously to some postulated combinations of intake target and decision rules than to others. Consider, for example, an experiment in which the array shown in figure 2c was the outcome and there were no independent indications of the position of the intake target. The simplest interpretation would be that the intake target lies on the 1:1 rail as depicted in the figure, and that the decision rule is 'feed until the nutritional target is at least achieved in both dimensions, regardless of an overshoot with respect to the nutrient present in the food in greater quantities'. A considerably more complex mathematical description would be required to reconcile this

outcome with an intake target postulated to lie elsewhere on the plane. Although mathematical complexity does not in itself necessarily detract from parsimony, any mechanistic or functional interpretation would have to rest on a greater number of assumptions than the mid-rail hypothesis and would on this criterion be less parsimonious.

3. APPLYING THE FRAMEWORK: INTAKE OF PROTEIN AND CARBOHYDRATE BY LOCUSTS

Raubenheimer & Simpson (1993) applied this framework to an investigation of the decision rules used by African migratory locusts (*Locusta migratoria*) in the regulation of protein and carbohydrate intake. Two hundred newly moulted fifth instar locusts, in a balanced factorial design experiment, were each given one of 25 chemically defined artificial foods containing either 7, 14, 21, 28 or 35% protein and one of the same levels of carbohydrates. A change in the level of either nutrient was compensated by an equivalent change in the non-nutritive bulking agent, cellulose fibre, allowing the concentration of all other nutrients

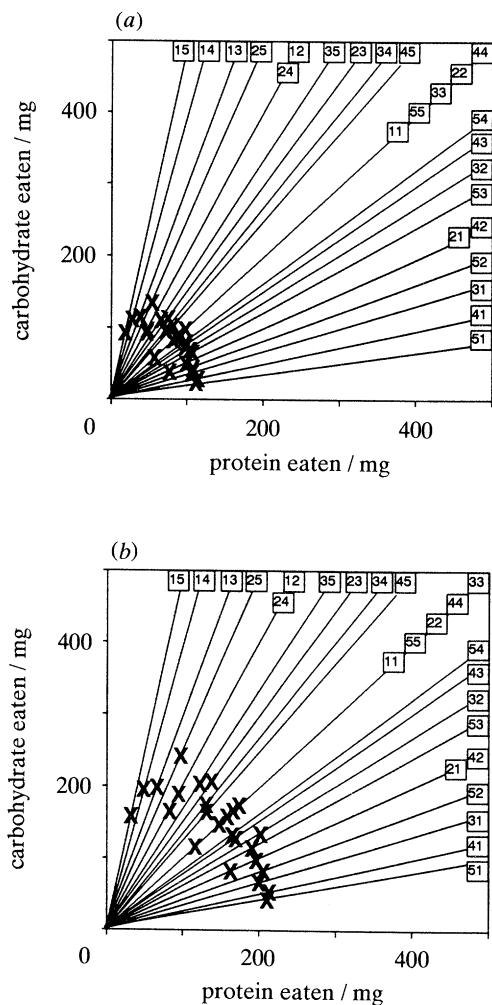


Figure 3. Nutritional planes from an experiment in which locust nymphs were provided with one of 25 artificial diets, containing one of five levels each of protein and digestible carbohydrate (7, 14, 21, 28 and 35%). The cumulative intake of digestible carbohydrate and protein is shown over (a) the first three days, and (b) the first five days of the fifth stadium. Each cross is the mean of eight locusts. Note the resemblance to figure 2*f*.

to remain constant among the diets. This gave a two-dimensional nutritional space with 19 rails, each representing a unique protein:carbohydrate ratio. Amounts eaten were measured for each insect after 3, 5, and 8 days and when the animal moulted out of the fifth stadium.

(a) Behavioural end points

Mean cumulative amounts of protein and carbohydrate eaten by each experimental group after 3 and 5 days are plotted in figure 3. The curved array of behavioural end points resembles most closely the rule of closest distance optimization depicted in figure 2*f*, assuming that the intake target relevant to this period is on or somewhere near to the mid rail. By day 8 the shape of the array had changed somewhat, with the behavioural end points on the outer rails beginning to curve away from the arc (figure 4). Over the full stadium, this process had developed further, with the

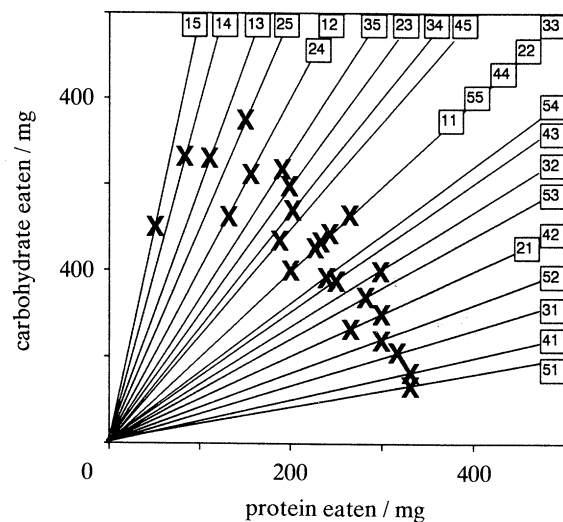


Figure 4. As for figure 3 but showing cumulative intake of protein and carbohydrate over the first eight days of the stadium. The arc apparent in figure 3 is beginning to straighten out as differences in development time appear (see text). This trend results in the arc bending outwards at the edges by the end of the stadium (see figure 5).

extremes having moved outwards to form arms on both sides of the arc, which is itself still discernible but has straightened somewhat (figure 5).

(b) Locating the intake target

As a first step towards estimating the position of the intake target relevant to the full stadium, the diets that resulted in low survival rates or increased development time were identified. Figure 6 shows that there were decreased survival rates and/or increased development times for animals on rails to the left of the rail for diets 1:2 and 2:4 and to the right of the rail for diets 2:1 and 4:2. The area delimited by these two rails was on this basis considered to be the

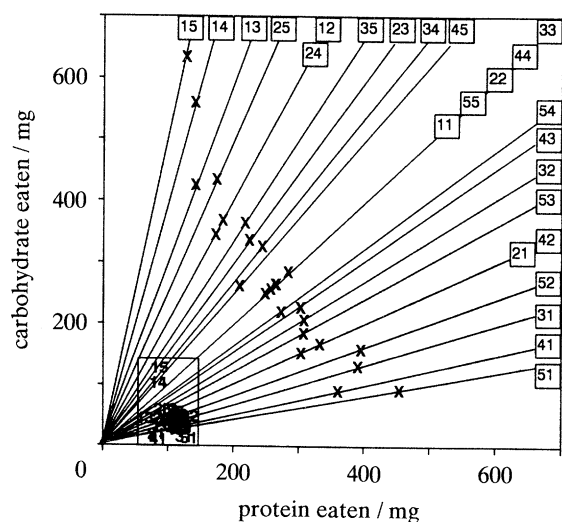


Figure 5. Intake of protein and carbohydrate over the entire stadium. The boxed insert shows protein and carbohydrate-derived growth on the same scale. Note the concentration of points indicating the position of the growth target. Arriving at this target has involved 'jumping' rails by differentially utilizing ingested protein and carbohydrate.

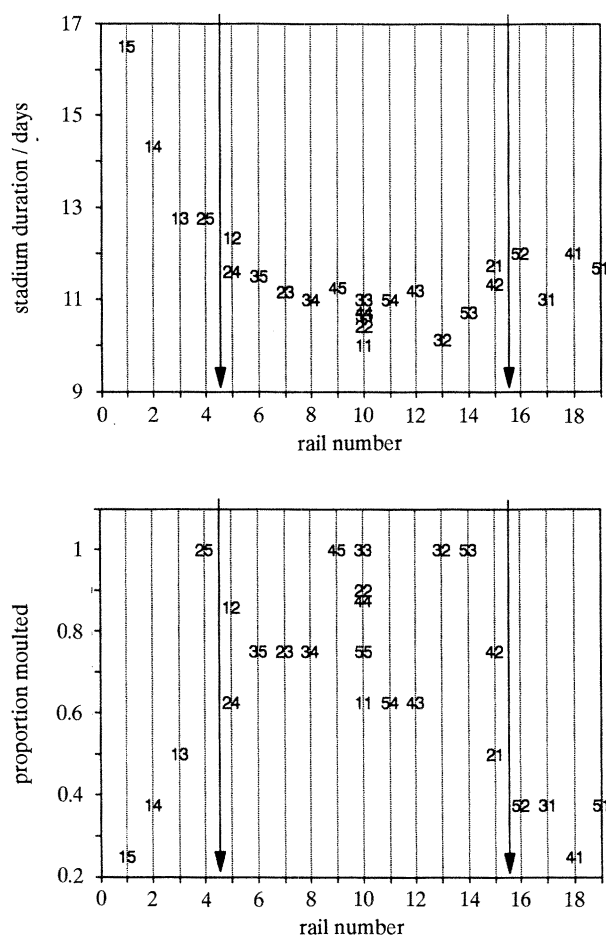


Figure 6. Plots showing stadium duration and proportion of locusts moulting to adulthood against rail number, where rails are numbered in increasing order of protein:carbohydrate ratio. The two-digit numbers indicate the position of the mean for eight locusts fed the food coded by that number (see figures 2 and 3). The arrows delimit the region within which locusts performed approximately equally well according to both criteria.

functional plateau likely to contain the intake target. Accordingly, behavioural and physiological evidence were sought, as described above, to further narrow the area likely to contain the intake target.

(i) *Behaviour*

A choice experiment was performed in which a group of ten insects were given a choice of diets 1:2 and 2:1, and a second group were given diets 2:4 and 4:2 (P. G. Chambers, unpublished results; Simpson *et al.* 1992). For the first group to reach the same behavioural end point as the second, they would have to eat twice as much food overall, but similar proportions of the two foods. Unequivocal defence of any putative intake target would, however, involve two groups of insects eating different proportions of the two choice foods. Therefore, two additional treatments were included, one providing a choice of diets 2:4 and 2:1, and the other a choice of 1:2 and 2:4. Figure 7 shows that the behavioural end points of all four experimental groups were closely clustered around a point between the 1:1 and the 4:5 rails. This demonstrates that locusts regulate both the total

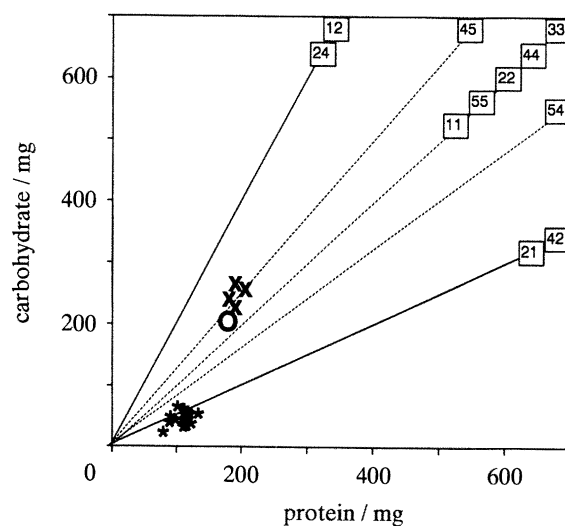


Figure 7. Nutritional plane with the position of the growth target indicated (cluster of asterisks, from figure 5) along with two independent estimates for the intake target. The crosses show the closely similar points reached by locusts provided with a choice of foods 1:2 or 2:4 with 2:1 or 4:2 (see Simpson *et al.* 1992). The circle indicates the target as estimated from the growth target and published values for metabolic costs and digestive asymmetries.

amounts of food eaten and the proportions of two alternative foods, and so achieve a similar behavioural end point, thus providing a strong indication of the protein and carbohydrate co-ordinates of the intake target.

(ii) *Physiology*

Figure 5 shows, plotted on the same scale as the intake points, the mean growth points for all groups of insects in the no-choice experiment. Considering the wide range of carbohydrate and protein eaten, the growth points clustered in a remarkably small area on the plane. The estimate of the position of the growth target was further improved by excluding the animals on rails outside of the performance plateau for developmental period and mortality. To estimate the nutritional target from the growth target, these growth points were corrected for respiratory costs of 150 mg carbohydrate, as measured for *Locusta* fed a choice of high quality natural foods (Clark 1957). We used the average utilization efficiencies for protein and carbohydrate from animals fed the 2:2, 4:4, 2:4 and 4:2 foods (Raubenheimer 1992) to estimate the intake target from the nutritional target, as these represented a range of foods within the region delimited by the performance plateau. Correcting the growth points for these values provided an estimate of the intake target which was remarkably similar to that obtained in the behavioural choice experiment (figure 7).

(c) *Interpretation*

Estimates derived from both physiology and behaviour suggested that the intake target for the full stadium lay close to the position in relation to the mid rail which would be expected if the curve-shaped arrays in figure 3 represented closest distance optimi-

zation. Therefore, assuming that the optimal rail was similar on days 3, 5 and over the entire stadium, the arrays suggest that the animals fed until they reached the point on their respective rails of least geometrical distance from the intake target.

What can be learned from this result about the proximate mechanisms regulating food intake in locusts? First, behaviour was not regulated so as to maximize the amount of food (and hence nutrients) ingested by each experimental group, for if this were so the square array shown in figure 2*a* would be the outcome. Neither did total nutrient (protein + carbohydrate) intake provide a reference point around which intake was regulated. If this was the case, without volumetric constraints, the slanted line depicted in figure 2*e* would be the outcome. An arc similar to that observed may result if total nutrient intake was the regulated variable, and there were volumetric constraints which prevented the animals on the outer rails from reaching the slanted line. This was not the case, as many of the animals on more central rails ate larger volumes of food than those on the outer rails (see Raubenheimer & Simpson 1993).

The closest distance arc demonstrates that, within a fixed feeding period (3 and 5 days), ingestive behaviour was the outcome of an interplay between mechanisms regulating protein intake and those regulating carbohydrate intake. The symmetry of the arc suggests that similar weighting was assigned to the commands emanating from these systems in the production of feeding behaviour, with the regulation of under and overeating being equally balanced for each nutrient group.

By the eighth day, the array had straightened somewhat, resembling more closely that depicted in figure 2*e*. Rather than assuming that protein and carbohydrate are functionally interchangeable (which is non-sensical in physiological terms), this development can be explained by considering the period spent feeding as a relevant independent variable. Feeding rate in fifth stadium *Locusta* is related to age within the stadium by an inverse U-shaped curve, peaking for locusts fed diets 2:2, 4:4, 2:1 and 1:2 at round the fifth day and reaching zero approximately two days prior to ecdysis as is also the case for locusts fed wheat (Simpson 1982). Insects on the outer diets had extended stadium duration relative to the others (figure 6*a*) so that the inverse 'U' was effectively shifted to the right for these animals. Therefore, by day 8 when the central animals on the plot began to stop feeding pending the moult round days 10 or 11, those on the outer rails continued to feed thus straightening the arc. The array for day 8 was, however, a transitory stage as evidenced by the distinct vertical and, to a lesser extent, horizontal appendages which had developed over the full stadium on either side of what remained of the original arc.

The shape of the final array can be interpreted to reflect the interplay of several regulatory responses. First, as discussed above, up to the fifth day of the stadium all animals used closest distance optimization. This enabled the animals on the more balanced foods to complete development and moult into adults within

10 to 11 days. Those forming the vertical arm (fed foods containing a low protein to carbohydrate ratio) had by this stage not yet reached the protein co-ordinate of the growth target, and so postponed the moult until this had been achieved. Effectively, this enabled additional protein intake through reducing the rate at which carbohydrate was ingested. The protein co-ordinate was, in fact, marginally exceeded, as could be predicted, considering the inherent constraints in the efficiency with which protein is utilized for growth. The animals forming the horizontal arm (fed foods containing a high protein to carbohydrate ratio) exceeded the carbohydrate co-ordinate of the growth target to a greater extent than those in the vertical arm exceeded the protein co-ordinate. This could be expected, as carbohydrate intake must provide for both structural and energetic requirements. It is, by contrast, highly unlikely that any protein would be oxidized to produce energy by the animals forming the vertical arm, as these animals had available an excess of carbohydrates.

Interestingly, while the growth target was exceeded to a greater extent by animals in the horizontal than those in the vertical arm, the opposite was true for protein and carbohydrate intake with respect to our estimate of the position of the nutritional target. The nutritional target was estimated to lie on the same protein co-ordinate as the growth target, but 150 mg higher on the carbohydrate axis, moving it approximately to 200 mg carbohydrate (see above). Both co-ordinates were exceeded by animals in the vertical arm of the array, but the maximum carbohydrate intake observed by animals in the horizontal arm was 120 mg (a deficit of 80 mg carbohydrate). A probable reason for this deficit is that amino acids in excess of the structural requirements of these animals could be deaminated and used in carbohydrate metabolism, thus reducing the requirements for dietary carbohydrates. By contrast, excess carbohydrates could not be converted to amino acids by animals forming the vertical arm, so their sole means of meeting their amino acid requirements was through ingestion of protein.

A striking result was that all groups approached the growth target by 'jumping' across rails through altering the efficiency of utilization of protein and carbohydrates. This can be seen in the tight clustering of growth points in figure 5, compared with the wide range in amounts of the two nutrients eaten. The increased clustering when animals outside of the performance plateau were excluded (figure 7) demonstrates that feeding on extreme foods did, however, affect growth. The relationship between amounts eaten of protein and carbohydrate and growth derived from these nutrient groups is plotted in figure 8. The difference in slope of the lines representing intake and growth is a measure of homeostatic ability. A notable aspect, is how the levelling of the growth slope, particularly for carbohydrates, breaks down on the extreme foods. The left hand side of the plot for carbohydrates is the most extreme in this respect, possibly providing an example of obesity in insects.

Finally, that some of the animals on even the most

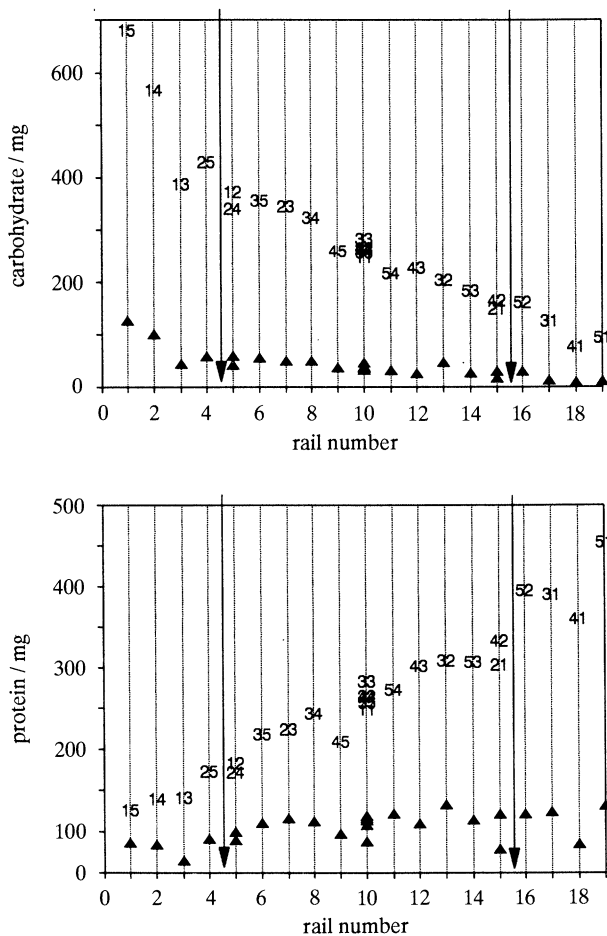


Figure 8. Plots of carbohydrate and protein intake and carbohydrate and protein-derived growth (triangles) against rail number. The flatness of the growth plots graphically illustrate the success of post-ingestive compensation, at least within the region delimited by the two arrows (see figure 7).

extreme rails moulted, demonstrates that it was possible to do so given a certain minimum intake of protein (vertical arm) and carbohydrate (horizontal arm). This may provide an explanation for the straightening of the arc for the full instar by animals on the performance plateau. As mentioned above, the straightening of the arc cannot be interpreted on physiological grounds to represent functional interchangeability of protein and carbohydrate up to the point when the minimal intake of protein and carbohydrate necessary to moult were achieved. However, further feeding beyond this point serves the function of laying down reserves for use in adult development. Such 'luxury storage' may not differentiate protein and carbohydrate but simply maximize intake of what is available in the food. Both protein and carbohydrate are used substantially during somatic and reproductive growth by adult *Locusta* (Chyb & Simpson 1990).

4. EVOLUTIONARY AND ONTOGENETIC ASPECTS

The geometric framework expounded above is generally applicable to all animal species and to all stages in the life history of a single species. In the following

sections we firstly explore ontogenetic effects and then illustrate how the framework can be used as a basis for comparative studies between species and life history traits. The comparative approach adds an extra dimension by allowing issues such as the generality of functional decision rules to be investigated, as well as enabling testing of specific evolutionary hypotheses.

(a) Ontogenetic effects

An important feature of intake, nutritional and growth targets is that they are not fixed but move over time within nutritional space. Much of this movement is ultimately driven by developmental programmes which alter the requirements of the tissues for nutrients and energy (Barton Browne 1993). For instance, as an animal grows, different body tissues grow at different times and rates in a developmentally determined sequence. Similarly, the production of reproductive tissues occurs at an appropriate time in the ontogenetic sequence. There are data from locusts and a few other insect species which allow the changing position of estimates of the intake target to be plotted. Two such examples are shown in figure 9. The first comes from the work of Cohen *et al.* (1987) on larvae of the cockroach, *Supella longipalpa*. Here larvae were provided with a free choice between two complementary foods, one lacking protein and the other digestible carbohydrate. If it is accepted that the selected ratio represents the intake target (see above and also next section), then, during the course of both the first and the last larval stadia, the target measured on successive 12 h periods moved progressively downwards towards the origin (as total food intake declined) and in so doing moved towards the mid-rail (i.e. the ratio P:C ingested increased).

The second example is from experiments by Chyb & Simpson (1990) on somatic growth in adult *L. migratoria*. Locusts were provided with two complementary foods, one containing one of a graded series of protein and the other one of a graded series of carbohydrate, and intake was measured every 24 h. A high P:C ratio was selected on the first day after ecdysis, and this fell progressively on subsequent days during the somatic growth phase as tissue growth declined. Total food consumption increased up to day 6, then declined, moving the daily target position outwards from the origin, then back again in a loop.

It remains to be seen whether decision rules controlling intake in situations where the intake target is unreachable vary with changes in the target's position during ontogeny, or whether the same rules apply with respect to the target wherever it lies in nutritional space. A related issue is dealt with in the next section: that of the commutability of decision rules between species.

(b) A comparative approach

In the case of fifth instar locust nymphs, the intake target for protein and digestible carbohydrate lies on the rail bearing foods containing a 46:54 ratio of P:C. Is this also true for other insects? Additionally, the

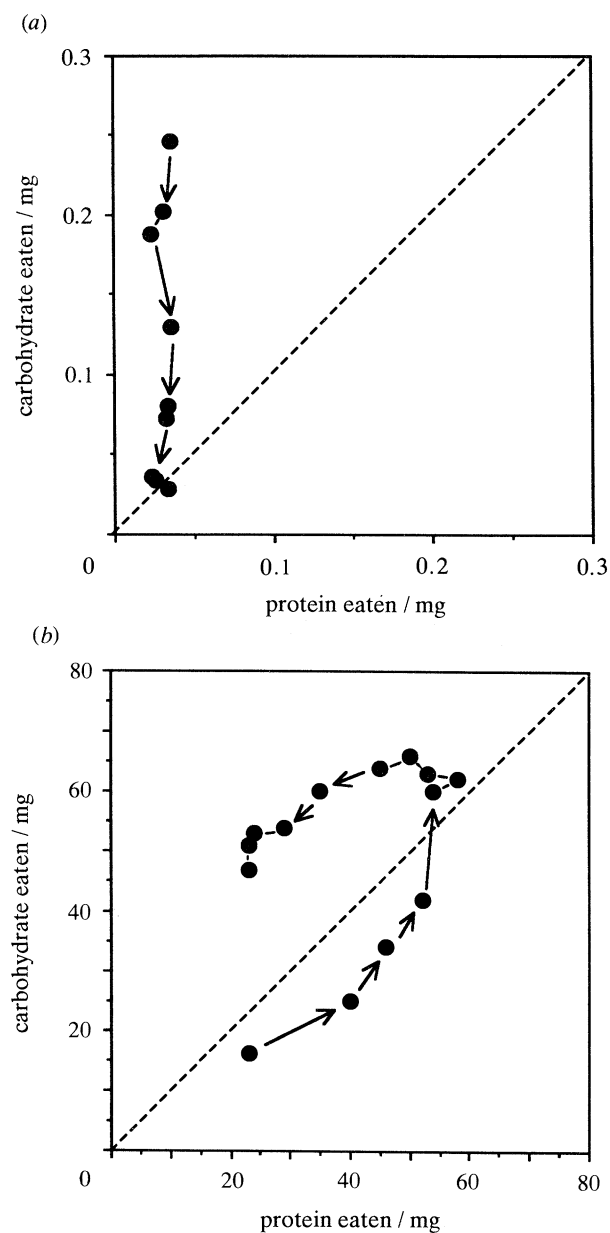


Figure 9. (a) Change during the first instar in the position of the intake target for protein and carbohydrate in *Supella longipalpa*, from data of Cohen *et al.* (1987). Symbols indicate the intake of protein and carbohydrate during consecutive 12 h periods across the stadium by larvae provided with a choice of complementary foods, one lacking protein and the other with no soluble carbohydrate. (b) Change in the position of the intake target during the somatic growth phase of adult development in *Locusta migratoria*, from data of Chyb & Simpson (1990). Data are from locusts given a choice of two foods, one containing one of three levels of protein and the other one of three levels of digestible carbohydrate. Symbols indicate intake on consecutive 24 h periods.

behaviour of locusts is consistent with closest distance optimization: does this rule also describe the behaviour of other species?

(i) *Closest distance optimization in other species*

Apart from *Locusta migratoria* there are data which allow a reasonable estimate of the position of the intake target to be made for the larvae of three other

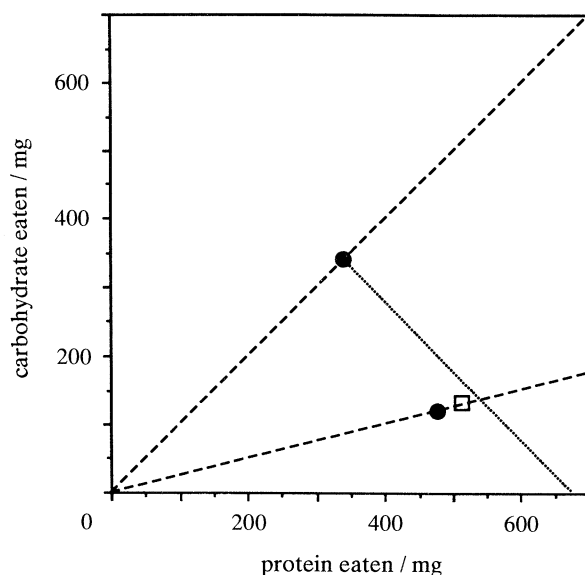


Figure 10. Intake of protein and carbohydrate by *Helicoverpa zea* across the last larval stadium, from data of Waldbauer *et al.* (1984). The open square represents the point reached by larvae given the chance to select between two complementary foods, one lacking only protein and the other lacking only soluble carbohydrate. This point lies on the 80:20 P:C rail. The closed circle on that same rail shows the point reached by larvae given a diet containing 80:20 P:C in a no-choice assay, while the closed circle on the mid-rail indicates the intake point for larvae on a 50:50 P:C diet with no choice. Note that the selecting larvae and those with an 80:20 diet reached almost the same point, and that a line drawn at right angles to the point reached by larvae on the 50:50 diet intersects the 80:20 rail close to the intake point of selecting larvae. If it is assumed that the selecting larvae reached the intake target, then this result is consistent with closest distance optimization.

insect species. These are the caterpillar, *Helicoverpa zea* (Waldbauer *et al.* 1984), the cockroach, *Supella longipalpa* (Cohen *et al.* 1988) and the beetle *Tribolium confusum* (Waldbauer & Bhattacharya 1973).

When larval *H. zea* were allowed to choose between two food blocks, one lacking protein and the other deficient in digestible carbohydrate they chose a ratio over the fifth stadium of 80:20 P:C, reaching a point with the co-ordinates 513 mg P, 133 mg C. If fed a food mixed in this same ratio in a no-choice assay the larvae came close to reaching this same point (478P, 120C) and performed (growth, development time) similarly well. When given a 50:50 food in a no-choice test they ingested 337P, 337C in one trial and 341P, 341C in another, again performing at close to the same level. Other foods used (100:0, 20:80) did not support growth nearly as well. Because the challenge provided by these foods was beyond the limits of the compensatory mechanisms of this animal, they are not relevant to the present discussion. If it is assumed that the selecting larvae, which had the entire nutritional plane available to them, reached their intake target, then the insects on the 50:50 food moved along that rail to a point which brought them very close to being the nearest geometric distance from the target (figure 10).

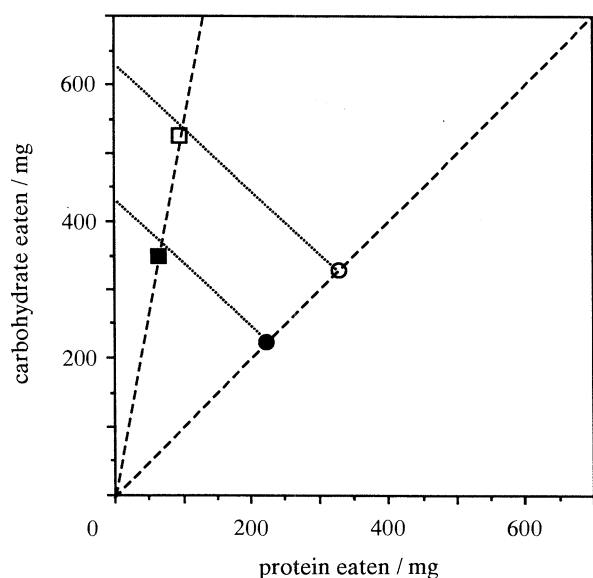


Figure 11. Intake of protein and carbohydrate by *Supella longipalpa* across the entire larval period (open symbols) and, in another experiment, across the last half of larval development (closed symbols), from the data of Cohen *et al.* (1987). The square symbols indicate the points reached by selecting nymphs and the circles are those reached by larvae given food with 50:50 P:C. Lines drawn at right angles to the latter points pass close to the intake of selecting insects, a result consistent with closest distance optimization if it is assumed that selecting larvae reached the intake target. Note how the selected ratio of P:C is much lower than for *H. zea* in figure 9.

When a similar experiment was performed on *S. longipalpa*, larvae selected a ratio of 16:84 P:C. Unlike the caterpillars, the cockroaches did not develop successfully if given a food mixed in the ratio they selected, although they performed as well as did the selecting larvae if given a 50:50 mix. This could be because the mixture, although optimal overall, did not reflect the changing needs of the larvae within stadia (see above; Cohen *et al.* 1987), or it could indicate that the selected intake was not the same as the intake target but resulted from the supernormal stimulus provided by the carbohydrate food (Simpson *et al.* 1988). Distinguishing these would require evidence that the point was defended in the face of nutritional challenges (see above). However, if it is assumed that the selected point did represent the intake target, then those larvae given a 50:50 food with no choice reached remarkably close to the nearest geometric distance from the target in two separate experiments (figure 11).

The chemically defined artificial foods listed in the compendium by Singh (1977) for rearing cultures of two other cockroaches, *Periplaneta americana* and *Blattella germanica*, are also strongly biased towards carbohydrate. Singh (1977) cites four studies on *P. americana*: Saksena & Perti (1970) tested eight foods and found that one with 32P:67C was best; Sieburth & McLaren (1953) successfully used 33:67, as did Wientjens (1972), whereas Fogash (1958) used 49:51. When data collected by Bignell (1978) for adult female *P. americana* fed foods varying in dextrin

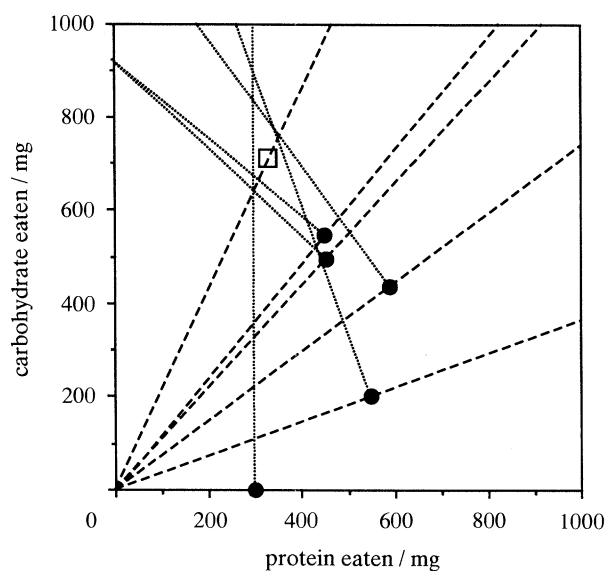


Figure 12. Intake of protein and carbohydrate by adult female *Periplaneta americana* given one of five artificial foods varying in dextrin content, from the data of Bignell (1978). Lines drawn at right angles to the intake points converge at a point (open square) on the rail 30:70. Other diet studies indicate that 30:70 is the best ratio for rearing this species and that the intake target is likely to lie somewhere close to that rail.

content are plotted and lines are drawn at right angles to the intake points on a P:C plane, these lines converge at a point which lies on the rail 31P:69C (figure 12).

A further study which warrants discussion is that of Waldbauer & Bhattacharya (1973) on larval *Tribolium confusum*. These insects were fed wheat germ, bran or endosperm, or else allowed to choose from a mix of all three. Larvae grew similarly well on germ or on the

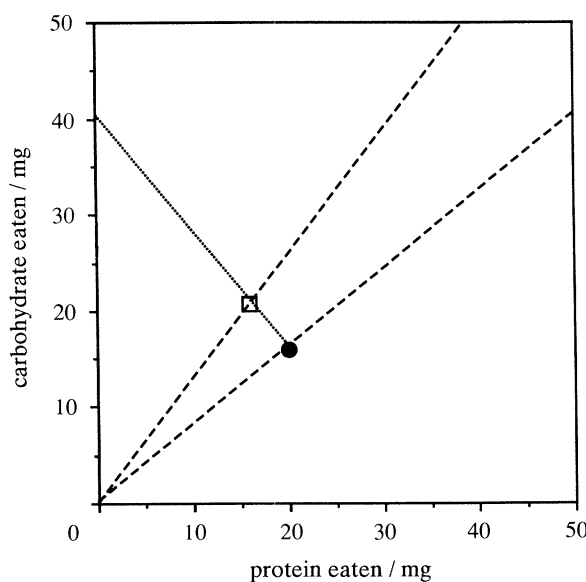


Figure 13. Intake of protein and carbohydrate by larval *Tribolium confusum* which were either allowed to select between wheat germ, bran and endosperm (open square) or else given only germ (closed circle). A line drawn at right angles to the circle passes through the square, which is consistent with closest distance optimization, assuming that the square represents the intake target.

Table 1. A listing of the species used in the comparative analysis of the effect of mycetocyte symbionts on P:C ratio required in the diet

(The column headed '% protein' contains the value: $P/(P+C) \times 100$, where P is the dry mass of protein and/or amino acids in the larval diet, and C is the dry mass of digestible carbohydrate. The column headed 'symbiont' indicates whether or not a species possesses mycetocyte symbionts. % Protein for each species was derived in large part from diet recipes listed in Singh (1977). Where better data for the position of the intake target were available, then these were used instead. This applies for *Supella longipalpa*, *Helicoverpa zea*, *Tribolium confusum*, *Locusta migratoria* and *Spodoptera littoralis*. Only chemically defined diets which supported growth and development to a level close to that for natural food were included. Where more than one such formulation was listed for a species the mean value from these was used. Additionally, diets which were chemically defined except for the addition of wheat germ were included in the data set, assuming protein and digestible carbohydrate contents for wheat germ of 25 and 20%, respectively (Waldbauer & Bhattacharya 1973).)

species	order	family	% protein	symbiont
<i>Calliphora erythrocephala</i>	Diptera	Calliphoridae	100	0
<i>Sarcophaga bullata</i>	Diptera	Sarcophagidae	100	0
<i>Cochliomyia hominivorax</i>	Diptera	Calliphoridae	99	0
<i>Phormia regina</i>	Diptera	Calliphoridae	98	0
<i>Cephus cinctus</i>	Hymenoptera	Cephidae	97	0
<i>Aedes aegypti</i>	Diptera	Culicidae	93	0
<i>Lucilia sericata</i>	Diptera	Calliphoridae	92	0
<i>Musca domestica</i>	Diptera	Muscidae	92	0
<i>Culex pipiens</i>	Diptera	Culicidae	83	0
<i>Drosophila melanogaster</i>	Diptera	Drosophilidae	83	0
<i>Diparopsis castanea</i>	Lepidoptera	Noctuidae	83	0
<i>Crambus trisectus</i>	Lepidoptera	Pyralidae	81	0
<i>Agria housei</i>	Diptera	Sarcophagidae	80	0
<i>Helicoverpa zea</i>	Lepidoptera	Noctuidae	80	0
<i>Itopectis conquisitor</i>	Hymenoptera	Ichneumonidae	77	0
<i>Exeristes roborator</i>	Hymenoptera	Ichneumonidae	75	0
<i>Anthrenus flavipes</i>	Coleoptera	Dermestidae	70	0
<i>Plodia interpunctella</i>	Lepidoptera	Pyralidae	69	0
<i>Aedes taeniorhynchus</i>	Diptera	Culicidae	67	0
<i>Stomoxys calcitrans</i>	Diptera	Muscidae	65	0
<i>Spodoptera littoralis</i>	Lepidoptera	Noctuidae	65	0
<i>Operophtera brunata</i>	Lepidoptera	Geometridae	63	0
<i>Delia antiqua</i>	Diptera	Anthomyiidae	62	0
<i>Sanninoidea exitiosa</i>	Lepidoptera	Sesiidae	61	0
<i>Delia platura</i>	Diptera	Anthomyiidae	60	0
<i>Chilo suppressalis</i>	Lepidoptera	Pyralidae	60	0
<i>Hypera postica</i>	Coleoptera	Curculionidae	58	0
<i>Porthetria dispar</i>	Lepidoptera	Lymantriidae	57	0
<i>Earias fabia</i>	Lepidoptera	Noctuidae	57	0
<i>Rhyacionia buoliana</i>	Lepidoptera	Tortricidae	57	0
<i>Rhyacionia frustrana</i>	Lepidoptera	Tortricidae	57	0
<i>Sphenophorus venatus</i>	Coleoptera	Curculionidae	56	0
<i>Scolytus multistriatus</i>	Coleoptera	Scolytidae	56	0
<i>Adoxophyes orana</i>	Lepidoptera	Tortricidae	56	0
<i>Diatraea grandiosella</i>	Lepidoptera	Pyralidae	55	0
<i>Dioryctria abietella</i>	Lepidoptera	Pyralidae	55	0
<i>Diabrotica undecimpunctata</i>	Coleoptera	Chrysomelidae	54	0
<i>Laodelphax striatellus</i>	Hemiptera	Delphacidae	54	0
<i>Hyphantria cunea</i>	Lepidoptera	Arctiidae	54	0
<i>Ostrinia nubilalis</i>	Lepidoptera	Pyralidae	54	0
<i>Xylocoris galactinus</i>	Hemiptera	Anthocoridae	53	0
<i>Lyctocoris beneficus</i>	Hemiptera	Anthocoridae	53	0
<i>Hemerocampa pseudotsugata</i>	Lepidoptera	Lymantriidae	53	0
<i>Laspeyresia pomonella</i>	Lepidoptera	Tortricidae	53	0
<i>Prionus imricornis</i>	Coleoptera	Cerambycidae	52	1
<i>Trichoplusia ni</i>	Lepidoptera	Noctuidae	52	0
<i>Dectes texanus</i>	Coleoptera	Cerambycidae	51	0
<i>Megacyllene robiniae</i>	Coleoptera	Cerambycidae	51	1
<i>Dermestes vulpinus</i>	Coleoptera	Dermestidae	51	0
<i>Estigmene acrea</i>	Lepidoptera	Arctiidae	51	0
<i>Anarstia lineatella</i>	Lepidoptera	Gelechiidae	51	0
<i>Malacosoma disstria</i>	Lepidoptera	Lasiocampidae	51	0
<i>Pseudaletia unipunctata</i>	Lepidoptera	Noctuidae	51	0

Table 1. *Continued*

<i>species</i>	<i>order</i>	<i>family</i>	<i>% protein</i>	<i>symbiont</i>
<i>Mamestra brassicae</i>	Lepidoptera	Noctuidae	51	0
<i>Agrotis orthogonia</i>	Lepidoptera	Noctuidae	51	0
<i>Euxoa auxiliaris</i>	Lepidoptera	Noctuidae	51	0
<i>Heliothis virescens</i>	Lepidoptera	Noctuidae	51	0
<i>Spodoptera frugiperda</i>	Lepidoptera	Noctuidae	51	0
<i>Agrotis ipsilon</i>	Lepidoptera	Noctuidae	51	0
<i>Pieris brassicae</i>	Lepidoptera	Pieridae	51	0
<i>Samia cynthia</i>	Lepidoptera	Saturniidae	51	0
<i>Hyalophora cecropia</i>	Lepidoptera	Saturniidae	51	0
<i>Hyalophora gloveri</i>	Lepidoptera	Saturniidae	51	0
<i>Synanthedon pictipes</i>	Lepidoptera	Sesiidae	51	0
<i>Celerio lineata</i>	Lepidoptera	Sphingidae	51	0
<i>Leptinotarsa decemlineata</i>	Coleoptera	Chrysomelidae	50	0
<i>Lema trilineata</i>	Coleoptera	Chrysomelidae	50	0
<i>Anthomus grandis</i>	Coleoptera	Curculionidae	50	0
<i>Xyleborus ferrugineus</i>	Coleoptera	Scolytidae	50	0
<i>Ahasversus advena</i>	Coleoptera	Silvanidae	50	0
<i>Reticulitermes flavipes</i>	Isoptera	Rhinotermitidae	50	0
<i>Pectinophora gossypiella</i>	Lepidoptera	Gelechiidae	50	0
<i>Prodenia eridania</i>	Lepidoptera	Noctuidae	50	0
<i>Prodenia litura</i>	Lepidoptera	Noctuidae	50	0
<i>Cadra cautella</i>	Lepidoptera	Pyralidae	50	0
<i>Ephestia elutella</i>	Lepidoptera	Pyralidae	50	0
<i>Epiphyas postvittana</i>	Lepidoptera	Tortricidae	50	0
<i>Schistocerca gregaria</i>	Orthoptera	Acrididae	50	0
<i>Locusta migratoria</i>	Orthoptera	Acrididae	46	0
<i>Attagenus megatoma</i>	Coleoptera	Dermestidae	45	0
<i>Hyllobius pales</i>	Coleoptera	Curculionidae	44	1
<i>Tribolium confusum</i>	Coleoptera	Tenebrionidae	43	0
<i>Bombyx mori</i>	Lepidoptera	Bombycidae	43	0
<i>Plagiohammus spinipennis</i>	Coleoptera	Cerambycidae	40	1
<i>Diatraea saccharalis</i>	Lepidoptera	Pyralidae	40	0
<i>Chrysopa carnea</i>	Neuroptera	Chrysopidae	40	0
<i>Melanoplus bivittatus</i>	Orthoptera	Acrididae	40	0
<i>Periplaneta americana</i>	Blattodea	Blattidae	37	1
<i>Oncopeltus fasciatus</i>	Hemiptera	Lygaeidae	37	0
<i>Trogoderma granarium</i>	Coleoptera	Dermestidae	35	0
<i>Tribolium destructor</i>	Coleoptera	Tenebrionidae	35	0
<i>Agrotaenia velutinana</i>	Lepidoptera	Tortricidae	34	0
<i>Lygus disponsi</i>	Hemiptera	Myridae	33	0
<i>Lasioderma serricorne</i>	Coleoptera	Anobiidae	28	1
<i>Tribolium castaneum</i>	Coleoptera	Tenebrionidae	28	0
<i>Blattella germanica</i>	Blattodea	Blattellidae	27	1
<i>Palorus ratzeburgi</i>	Coleoptera	Tenebrionidae	20	0
<i>Tenebrio molitor</i>	Coleoptera	Tenebrionidae	20	0
<i>Ephestia kuehniella</i>	Lepidoptera	Pyralidae	20	0
<i>Neomyzus circumflexus</i>	Hemiptera	Aphididae	18	1
<i>Stegobium paniceum</i>	Coleoptera	Anobiidae	17	1
<i>Sitophilus oryzae</i>	Coleoptera	Curculionidae	17	1
<i>Supella longipalpa</i>	Blattodea	Blattellidae	16	1
<i>Sitophilus granarius</i>	Coleoptera	Curculionidae	16	1
<i>Sitophilus zeamais</i>	Coleoptera	Curculionidae	16	1
<i>Oryzaephilus surinamensis</i>	Coleoptera	Silvanidae	16	1
<i>Myzus persicae</i>	Hemiptera	Aphididae	14	1
<i>Toxoptera aurantii</i>	Hemiptera	Aphididae	14	1
<i>Aphis fabae</i>	Hemiptera	Aphididae	14	1
<i>Macrosteles fascifrons</i>	Hemiptera	Cicadellidae	12	1
<i>Acyrtosiphon pisum</i>	Hemiptera	Aphididae	11	1
<i>Macrosiphum euphorbiae</i>	Hemiptera	Aphididae	11	1
<i>Schizaphis graminum</i>	Hemiptera	Aphididae	11	1
<i>Rhopalosiphum maidis</i>	Hemiptera	Aphididae	10	1
<i>Macrosiphum avenae</i>	Hemiptera	Aphididae	10	1
<i>Planococcus citri</i>	Hemiptera	Pseudococcidae	6	1

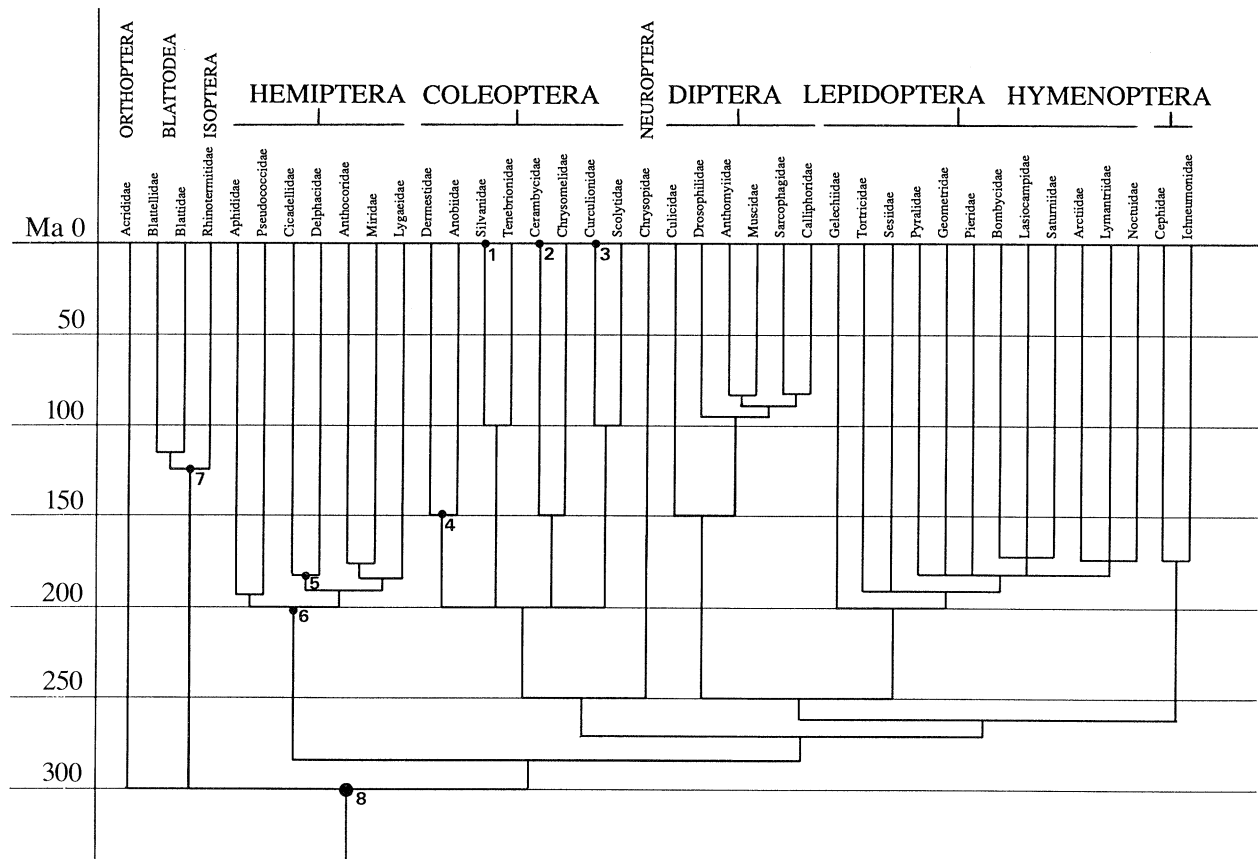


Figure 14. The phylogeny used for the comparative analysis of the effect of possession of mycetocyte symbionts on P:C ratio required in the diet. Small circles represent nodes where phylogenetically independent contrasts were possible. The large circle at the root node of the phylogeny was a point of independent contrast if the single species of termite was removed from the phylogeny. Numbering of nodes refers to table 2.

selected mix. Using data on the protein and carbohydrate content of the foods (Waldbauer & Bhattacharya 1973) it is possible to plot the intake points on a P:C plane. If it is assumed that the selecting insects reached their intake target, then the larvae given only germ moved along their rail to the point closest to the target (figure 13).

Although *L. migratoria*, *H. zea*, *S. longipalpa*, *P. americana* and *T. confusum* appear to differ widely among each other in the position of their intake target for protein and carbohydrate, their responses in no-choice assays when confined to foods that do not allow the target to be reached are all consistent with the functional rule 'move to the nearest geometric distance to the target'. Although tentative, this is a most interesting conclusion which warrants more rigorous investigation.

(ii) *A comparative analysis: the role of mycetocyte symbionts*

Although there are few studies which enable reasonable estimation of the position of the intake target, there is an extensive literature on the development of successful artificial foods for a wide range of insect species. Formulation of an artificial food generally occurs after testing a series of alternative recipes, with the best at supporting growth and development being ultimately chosen for rearing the culture. This means that the ratio of, for instance, protein/amino acids to digestible carbohydrate, will probably not be too far

from the ratio which defines the rail bearing the intake target. It is unlikely, however, given both the limited number of alternatives tested in the development of most artificial foods and the compensatory abilities of insects, that the rail which represents the P:C ratio in the food passes through the intake target. However, the fact that a food supports reasonable growth means that the ratio of protein/amino acids to digestible carbohydrate it contains must fall somewhere within the range of ratios which defines the compensatory limits of the insect, i.e. the performance plateau (see above), and encompasses the intake target. Admittedly, in the case of locusts this is a large range (from 33:67 to 67:33). However, differences between species in P:C ratios of their customary artificial foods might indicate differences in the slope of the rail bearing the intake target. Before proceeding with such an investigation, two other potential problems should be considered. Firstly, the artificial foods for rearing insects throughout their larval stadia are almost invariably homogeneous and unchanging in nutritional composition, and so do not reflect ontogenetic changes in requirements throughout larval development (see previous section). Instead, they are a compromise which successfully accommodates ontogenetic changes and, at best, indicates the integrated position of the intake target across larval development. Secondly, the formulation of artificial foods has not occurred independently for every species. In many

Table 2. Table summarizing the change in the ratio of dietary protein/amino acids to soluble carbohydrate for the phylogenetically independent contrasts shown in figure 15

(At each of the eight nodes there was a decline in % P when taxa containing mycetocyte symbionts were contrasted with taxa lacking such organisms. The eighth nodal contrast is at the root of the phylogeny and is only valid if the single species of termite is omitted from the rest of the analysis. For nodes 1–7, $p=0.0023$ when the data are compared in a t -test against a population mean of zero. The analysis was performed using branch lengths in the phylogeny derived from the nodal ages shown in figure 14. When fixed branch lengths are used the result is similar, with all eight nodes giving negative values and a mean of -26.7 ($p=0.0025$).

node number	change in % P at node
1	-34.0
2	-3.3
3	-31.4
4	-27.7
5	-42.0
6	-32.0
7	-23.2
8	-12.3
<hr/>	
mean change in % P (excluding 8):	-27.7

cases, species are reared in the laboratory on foods which were modified from ones already developed for related species. This is particularly apparent for Lepidoptera and aphids (see Singh 1977).

With all of the above in mind we have used the extensive list of artificial foods compiled by Singh (1977) to derive a list of P:C ratios for 117 species (table 1). Changes and augmentations to Singh's list are detailed in the caption to table 1. There is an extraordinary range of ratios, from 100:0 to 6:94. Encouragingly, the ratios listed for other cockroaches, acridids and some caterpillars and beetles are not dissimilar to the more detailed and convincing studies discussed above.

Such a data set provides an opportunity to test specific hypotheses relating to the physiology and life history of different species. For instance, in recent years there has been an increasing interest in the nutritional role of mycetocyte symbionts in insects. There is evidence in some insects, namely certain Homoptera, Blattodea and Coleoptera, that such microbial associations are important to the nitrogenous nutrition of the hosts (Buchner 1965; Douglas 1989, 1992). It might therefore be predicted that insects possessing mycetocyte symbionts would have a lower P:C ratio than those without. Listed for each species in table 1 is whether or not the insect possesses mycetocyte symbionts. Attributions were based on the reviews of Buchner (1965) and Douglas (1989, 1992, personal communication). A cursory glance at the data seems to confirm the prediction, but before it is possible to make such a claim it is necessary to carry out an appropriate comparative analysis.

It is not valid to treat all species as statistically

independent points as related species may well share the same traits by descent, rather than having evolved them independently (Harvey & Pagel 1991; Harvey & Purvis 1991). As discussed above, related species may also tend to share the same artificial food because of the historical sequence in which diets were developed by entomologists. To overcome such problems we have chosen to use the statistical procedure developed by Purvis (1991), which was based on the work of Felsenstein (1985, 1988) and Burt (1989). The null hypothesis is that changes in the dichotomous character 'mycetocyte symbionts' (present or absent) do not influence the ratio of protein/amino acids to digestible carbohydrates required in the diet. The programme only considers phylogenetically independent contrasts between species differing in the character trait. Species used in a contrast are not used again for contrasts at higher levels in the phylogeny. When all the taxa at a node share the same character condition, a representative value for the node is calculated and the entire node is compared with another node or taxon possessing the other character trait. Only one contrast is made at each node, the taxa at a node being split into two groups according to their character state. Further details of the method are outlined in Purvis (1991).

The phylogeny used in the analysis is shown in figure 14. It is based on Crowson (1981), Carver *et al.* (1991), Hennig (1981), Kristensen (1991), Nielsen & Common (1991) and A. C. Pont (pers. comm.). Approximate nodal ages were derived from Hennig (1981) and Kucolova-Peck (1991). The results from the analysis are shown in table 2.

It is clear that in each of the seven independent contrasts the acquisition of mycetocyte symbionts was accompanied by a reduction in the P:C ratio. This was statistically significant in both a t -test and a sign test. The result was also robust to changes in estimates for branch lengths in the phylogeny: it made no qualitative difference whether or not all branch lengths were set to the same value or whether they were calculated according to estimated nodal ages.

The first contrast was within the Silvanidae, between *Oryzaephilus surinamensis*, a member of the only genus in the Family to possess mycetocyte symbionts (Douglas 1989), and the foreign grain beetle, *Ahasversus advena*. The second was between the weed cerambycid, *Dectes texanus*, and a group of three wood-boring species in the same Family. Mycetocyte symbionts are not found in cerambycids that 'develop in fresh leaf wood and in plants' (Buchner 1965, p. 804), whereas they occur in wood-boring species. The third nodal comparison was within the Curculionidae between two plant-feeding species (the boll weevil and the zoysiagrass billbug) and three wood and grain-feeding species. The fourth contrast was made between representatives of the families Dermestidae (no mycetocyte symbionts) and the Anobiidae. The fifth comparison was between the delphacid bug, *Laodelphax striatellus*, which lacks mycetocyte symbionts (although it has intercellular yeasts (Douglas 1989)), and the cicadellid, *Macrostelus fascifrons*. Contrast number six was between four mycetocyte-free

heteropterans (two predatory anthocorids, the seed-eating *Oncopeltus fasciatus*, and the mesophyll-feeding mirid, *Lygus disponsi*) and the phloem-feeding aphids plus a pseudococcid. The final comparison was made between the Blattodea and a termite, *Reticulitermes flavipes*. The termites have apparently lost their mycetocyte symbionts since diverging from the Blattodea (see Douglas 1989). If the termite is removed from the data set, then this final contrast falls back to the root of the phylogeny, between cockroaches and all other species not so far contrasted, all of which lack mycetocyte symbionts. This group includes all representatives of the orders Neuroptera, Diptera, Lepidoptera and Hymenoptera, as well as the remaining Coleoptera. The change in % P at the root node is, like the seven other contrasts, negative with the acquisition of mycetocyte symbionts (a difference at the node of -12.3% protein).

Such an analysis provides the basis for laboratory experiments to test the hypothesis that possession of mycetocyte symbionts causes a change in the position of the intake target. In addition, there are various other hypotheses which could be tested in the same way. For instance, slow growing insects should have lower P:C ratios than fast growing ones; insects that feed mainly as larvae should have higher P:C ratios than those that feed extensively as adults; P:C ratios should be higher for more than for less fecund species, and so on.

5. PHYSIOLOGICAL MECHANISMS

So far we have outlined the conceptual framework in terms of functional responses, and shown how ontogenetic and comparative studies fit readily into this framework. During recent years considerable attention has been given to the physiological systems controlling the regulation of nutrient intake in insects, particularly the locust (e.g. Simpson & Simpson 1990, 1992; Simpson *et al.* 1991; Friedman *et al.* 1991; Simpson & Raubenheimer 1993). The evidence indicates that the blood is central to the control of nutrient intake, with titres of nutrients (amino acids and sugars in particular) and other correlated parameters such as osmolality providing feedbacks onto food location, selection and ingestion. The blood provides a summary of the current nutritional state of the animal. The linkage with behaviour involves both learning (Simpson & White 1990; Champagne & Bernays 1991) and direct feedbacks. The best known of the latter is a direct modulation of the sensitivity of mouthpart taste receptors which respond to nutrients in the food, notably amino acids and sugars, by those same nutrients in the blood (Simpson *et al.* 1991; Simpson & Simpson 1992). The taste receptors act as differential detectors, their threshold to external stimulation from food being set by blood nutrient levels, which in turn reflect the nutritional state of the insect.

6. THE PROBLEM OF SCALING

(a) General considerations

An important issue that remains to be discussed is the

problem of scaling. It is easy to see that the geometric patterns we have used to interpret decision rules are highly dependent upon the scaling of the axes bounding the nutritional space. By scaling, we do not mean the question of whether the axes are represented on, for example, linear versus logarithmic scales, for any such transformation to both axes preserves the underlying relationships and merely describes them in different terms. Rather, each nutrient group has several properties (such as mass, molarity, energy content, etc.) and conversions among these may influence the two axes differently. For example, an array based on the dry mass of two nutrient groups eaten will look different when plotted on a molar scale if the nutrients differ in molecular mass. Whereas such effects are not of particular consequence for interpreting arrays like those in figure 2*a-d*, where regulation of the two nutrients is orthogonal, they are of considerable importance in cases where regulatory mechanisms for the two nutrients interact (e.g. figure 2*e,f*).

Because decision rules are a property of the animal under study, it is important to aim at using axes that are scaled in a way that is functionally relevant to that animal. The problem is, how does one determine the appropriate scaling?

An experiment in which intake of two nutrients is recorded across a range of foods will provide a data set in which points have a particular ordinal relationship to each other. This property of the data is independent of the scaling of axes: scaling simply adjusts the distance between points, not their ordinal positions. Providing certain ordinal properties are met, the data set will be scalable so that it can be transformed into a geometric pattern which describes a particular functional rule. Using different scaling there may be instances in which the same data set is transformable into patterns representing other functional rules.

If, because of the ordinal properties of the data set, it is only possible to transform it into one of the functional rules under investigation, then this simultaneously provides support for the verity of the rule and of the scaling. This is what McFarland & Houston (1981) called the 'conjoint measurement approach' in their state-space model of motivation. If, however, the data set is transformable into more than one functional rule, then the only way to distinguish between these is by investigating the biological meaningfulness of the different scaling relationships. This requires some knowledge of the physiology of the animal under investigation and of the biochemistry of the foods being eaten. Choosing which scaling is functionally meaningful then becomes an interesting question in its own right and provides a basis for further experimentation.

(b) Scaling of protein and carbohydrate axes for locusts

Our interpretation of the results from locusts and other insects has been based on plotting protein and carbohydrate intake in units of dry mass. However, because carbohydrates and proteins are absorbed from the gut after digestion in the form of monosac-

charides and amino acids respectively, it seems that more appropriate units ought to be the total numbers of such molecules ingested, both in free form (if these occur in the food), and as the digestible constituents of the protein and carbohydrate present in the food. Plotting intake in such units would distort the geometric patterns found when dry mass is used because most amino acids have a lower molecular mass than do monosaccharides. Because each milligram of protein eaten contains more amino acid molecules than there are molecules of monosaccharide in an equivalent mass of sucrose and starch, this means that the protein axis is expanded relative to the carbohydrate axis, as compared with plots using units of dry mass.

This, however, assumes that every molecule ingested is of equal utility to the insect. Although it is the case that all monosaccharide molecules are of equal potential usefulness, the same is not true for amino acids. The problem is that, although digestible carbohydrate represents a single axis, protein is a composite of 20 or so different axes, one for each of the protein amino acids. Combining the latter into one axis means that essential amino acids are combined with non-essentials, and even within these two categories not all amino acids are of equal usefulness to the insect. A proportion of ingested amino acids will be of little or no value. Adjusting the scaling of the protein axis to account for such variation will involve contracting the protein relative to the carbohydrate axis. This will counteract the expansion caused by using molecules rather than dry mass ingested.

If it happens that the adjustment due to a proportion of the amino acid intake being useless is equal to the proportional difference in mass between amino acid and monosaccharide molecules, then the same geometric patterns will emerge as found upon plotting intake in terms of dry mass. Because the mean molecular mass of amino acids in the protein mix used in the locust diets is 130 (from data in Abisgold & Simpson 1987), as compared with 180 for glucose, a dry mass scale would be appropriate if *ca.* 30% of the total amino acids in the protein mix are effectively non-nutritional.

As discussed earlier, the geometric patterns of intake found for several species of insect, with widely differing intake targets, all appear to be consistent with closest distance optimization. If this stands up to more rigorous investigation it could be used as evidence for dry mass providing close to the correct scaling of the two axes, at least for the species tested and the proteins used in their foods. Changing the scaling by plotting molecules of monosaccharide ingested against total amino acid molecules eaten (ignoring any heterogeneity in the utility of different amino acids) provides a geometric pattern which is impossible to reconcile with what is known of the biology of the locust, and also completely destroys any consistency in pattern between species.

It is difficult to ascertain experimentally what proportion of dietary protein is of high utility. A potentially powerful approach would be to test a range of dietary proteins with different profiles of amino acids or with amino acid supplementation, and

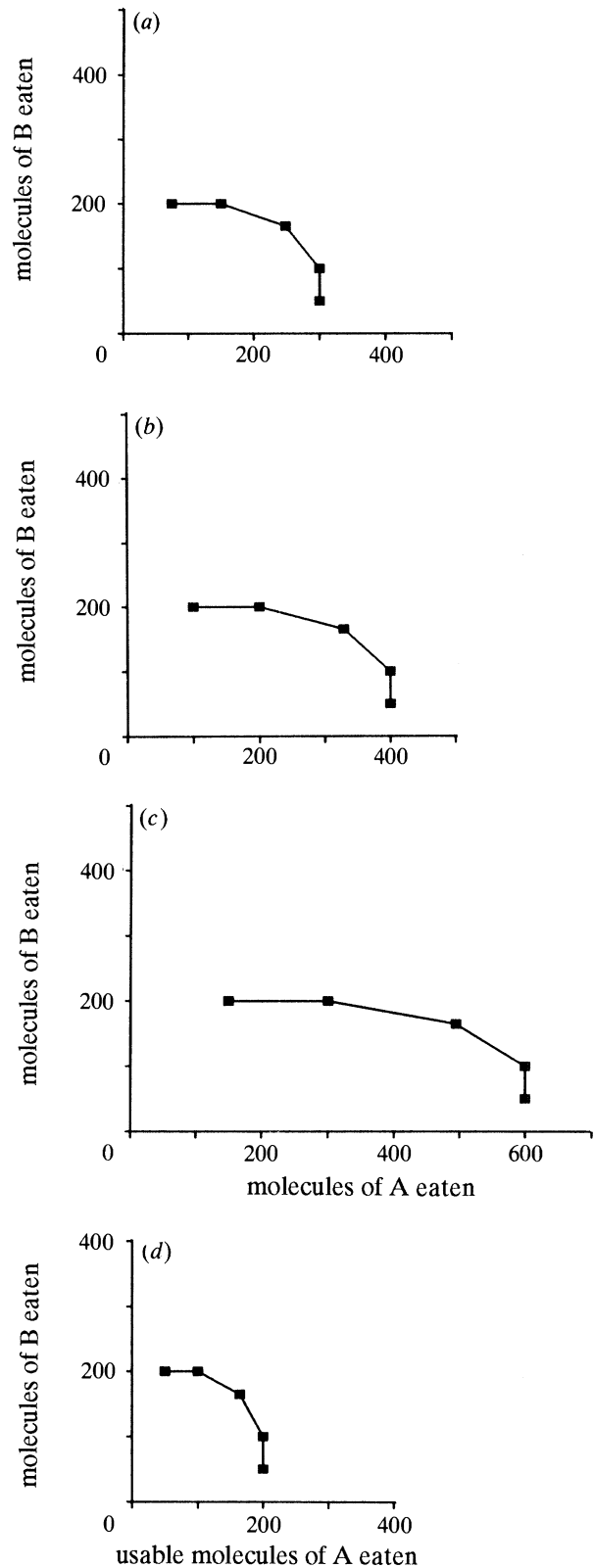


Figure 15. Nutritional planes plotting intake of nutrients A and B. (a) Food 1; (b) food 2; (c) food 3; (d) foods 1–3. Nutrient A is a compound nutrient, composed of four constituent molecules, a, b, c and d, of which only a and b are usable by the insects. Foods 1–3 each contain nutrient A with a different blend of a:b:c:d (2:2:1:1 for food 1; 2:2:2:2 for food 2 and 2:2:4:4 for food 3). The bottom graph shows how three apparently different arrays are united into a common pattern when intake is plotted in the correct units (molecules of a + b eaten, rather than total molecules of A).

to look for a scaling unit which unifies all outcomes, i.e. which produces an intake array which is consistent across all experiments (figure 15). Such an approach is much easier to apply when dealing with an animal, such as an aphid, whose food contains all its nitrogenous nutrients in the form of free amino acids.

Although dry mass may, fortuitously, provide an appropriate scaling for intake of protein and carbohydrate, it clearly would not be appropriate for all nutritional axes. When micro-nutrients are considered the units ought really to be molecules ingested.

7. DISCUSSION

We have presented an empirical framework for nutrition. The experimental testing of the framework has involved insects, but in principle it is applicable to all animals. In addition, plant nutrition shares many features with that of animals and is appropriate for such a geometric analysis. The multidimensional nature of plant nutrition has been considered in the models of Tilman (1988) and Gleeson & Tilman (1992). An explicitly geometrical approach has also been adopted by livestock nutritionists when considering food mixtures (Moon & Spencer 1974; Parks 1982; Emmans 1991).

There have been three main approaches to investigating the causal mechanisms of feeding and nutrition in animals. First, mechanistic studies have measured behavioural responses of animals to the manipulation of one, or a few (e.g. Raubenheimer & Simpson 1990; Raubenheimer 1992), independent variables. Second, systems theory has been used to study the interactions that take place among the mechanisms underlying behaviour (McFarland 1971; McFarland & Houston 1981). Third, behavioural ecologists have used evolutionary principles as a starting point to predict behaviour (Kamil *et al.* 1987; Hughes 1990).

Recently, there has been a tendency for behavioural ecologists to acknowledge the value of integrating mechanistic aspects into the formulation and interpretation of empirical tests of such predictions (Barlow 1989; Krebs & Kacelnik 1991; Hughes 1993). In this paper we have introduced a research framework that aims to integrate mechanism and function from the other direction, having as a baseline the state-space systems approach to the study of mechanisms and from there working towards function. The point of integration is in two major respects more than a post-hoc functional interpretation of mechanistic phenomena (Gould & Lewontin 1979; Pierce & Ollason 1987). First, the framework aims explicitly to identify functional optima (intake, growth and nutritional targets) via investigations of the design features of evolved homeostatic mechanisms. Calow (1976) has presented an excellent discussion of the relevance to functional interpretations of studying the design features of physiological mechanisms. Second, the incorporation of a performance axis provides an empirical link to functional consequences of different behavioural and physiological responses. In contrast to behavioural ecology, our approach is thus in emphasis

primarily an empirical one, in which experiment precedes theory.

There are two levels at which our framework considers the adaptiveness of behaviour: the position of the target, and the response of the animals when constrained from reaching the target. It might be predicted that the position of the nutritional target would, in evolutionary time, be influenced by ecological factors. We have demonstrated how hypotheses regarding such ecological influences may be tested using the comparative method. Likewise, clearly formulated, testable hypotheses may be made about changes in the position of the targets with ontogenetic development. It might, for example, be predicted that the required protein:carbohydrate ratio would decrease when an animal ceases growing and feeds predominantly for energy metabolism, or that the ratio would increase as an adult female approaches reproductive maturity. The position of the target may also move in ecological time, as when a homeothermic animal has increased energy requirements during winter or when different populations of a species occur at different latitudes.

Approaching the target is a problem of homeostasis. In most ecological situations, there will be times at which the animal is constrained from reaching the intake target. This may be due, for example, to fluctuations in food availability (or food availability which is constant when developmental changes have resulted in a shift in the position of the target). Likewise, feeding time, or the availability of a particular resource, may be restricted due to biotic (predation, mate finding, etc.) or abiotic (e.g. thermoregulatory) factors (see Raubenheimer & Bernays (1993) for an example in grasshoppers). In such cases the animal may regulate intake so as to reach the point of best compromise, and also regulate post-ingestive processes to further minimize the costs of the constraints. The point of best compromise is, like the position of the target, itself likely to be subject to natural selection and can thus also be investigated using the comparative method. In our study, all the insect species examined appeared to use closest approach optimization when restricted from reaching the protein and carbohydrate co-ordinates of the intake target. This may not be the case for all nutrients, or for other animals. For example, Trumper & Simpson (1993) found that locusts regulate salt intake against intake of all other nutrients if given the choice, but when constrained from doing so, respond as shown in figure 2*b* (where the *y*-axis represents salt intake and the *x*-axis, intake of other nutrients).

An important consequence of defining our targets as functional optima, is that peaks in the performance dimension should correspond with the estimates of the position of the targets derived from mechanistic studies. If this is not the case, it is likely that some crucial dimension has been omitted from the model or that the performance variable is not a satisfactory measure of fitness. For example, an animal may over a given time period regulate protein to a position on the protein-carbohydrate plane and yet perform better when restricted to a region lower on the protein axis, if

the experimental diet lacks some factor necessary to protein metabolism. It may, similarly, be that the laboratory conditions exclude some important ecological cost to reaching the apparently superior position. For example, most plants in an herbivore's habitat with high protein content may also contain nitrogen-based toxins (Bryant *et al.* 1983). While these examples are hypothetical, they are included to illustrate that instances where the animal's behaviour does not maximize its performance, if they occur, may give rise to testable nutritional and ecological hypotheses.

For simplicity, the targets have thus far been considered as static points in nutritional space. However, the ontogenetic studies we have presented show very clearly that the targets may move with time, and can thus more realistically be viewed as trajectories. This adds an additional dimension, time, to the within and between-species comparisons discussed above. It is, for example, possible that integrated over the life-cycle two species have a similar nutritional target, but developmental differences determine that the route this travels from birth to death differs. Such instances would give rise to interesting questions about both mechanisms and selective pressures, which can be clearly stated and tested within our framework.

In addition to integrating function and mechanism, we have demonstrated that the framework accommodates ontogenetic and comparative aspects. Integration of these four levels of explanation has long been considered to be one of the main aims of ethology (Tinbergen 1963), but there are few instances where this has been achieved (Dawkins 1989). We consider its potential for uniting empirically and theoretically within a single framework all facets of nutritional behaviour to be a major advantage of our schema.

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