

Integrative models of nutrient balancing: application to insects and vertebrates

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Abstract

We present and apply to data for insects, chickens and rats a conceptual and experimental framework for studying nutrition as a multi-dimensional phenomenon. The framework enables the unification within a single geometrical model of several nutritionally relevant measures, including: the optimal balance and amounts of nutrients required by an animal in a given time (the intake target), the animal's current state in relation to these requirements, available foods, the amounts of ingested nutrients which are retained and eliminated, and animal performance. Animals given a nutritionally balanced food, or two or more imbalanced but complementary foods, can satisfy their nutrient requirements, and hence optimize performance. However, animals eating noncomplementary imbalanced foods must decide on a suitable compromise between overingesting some nutrients and underingesting others. The geometrical models provide a means of measuring nutritional targets and rules of compromise, and comparing these among different animals and within similar animals at different developmental stages or in different environments. They also provide a framework for designing and interpreting experiments on the regulatory and metabolic mechanisms underlying nutritional homeostasis.

Introduction

An animal's metabolic systems require several nutrients simultaneously, each ideally at its individual optimal level. At the same time most foods consist of several, if not numerous, different chemical compounds, the relative levels of which can vary substantially even among different items of the same food type, or within a single food item at different times. Nutritional regulation is therefore a complex problem of balancing the uptake and loss of a number of compounds simultaneously against multiple, and changing, metabolic requirements.

While natural and artificial selection have, it appears, been competent at solving such problems from the animals' viewpoint, the complexity of nutritional systems remains a challenge to researchers. A conceptual and experimental approach is needed which reduces to tractable parameters the numerous interactions among the components, while retaining the biologically salient features of the system. One approach, most explicitly articulated in optimal foraging theory (Stephens & Krebs, 1986), has been to assume that a single nutritional cur-

rency—usually energy—is sufficiently important from the animal's perspective to characterize nutrition on its own (e.g. Ydenberg *et al.* 1994). An elaboration of this has been the recognition that constraints might be imposed on energy intake by the animal's requirements for non-energetic nutrients, such as mineral salts, water or nitrogen (Pulliam, 1975; Belovsky, 1990).

The unidimensional approach of optimal foraging theory has provided an important focus for unifying a range of areas in nutritional research (e.g. physiology, psychology, ethology, ecology, evolutionary biology—Hughes, 1993), but its simplicity comes at the cost of nutritional realism. In assuming the primacy of energy, it does not provide a basis for investigating the ways that animals *actually* weight the relative importance of the various food components. An important step in this direction has been the use of mixture theory to characterize the composition of foods (Moon & Spencer, 1974; Parks, 1982; Emmans, 1991). However, while dealing elegantly with the multi-dimensionality of foods, such models do not easily extend to a description of the interaction of the animal with foods (Emmans, 1991). One reason for this is that individual nutrients are represented in mixture theory as a percentage of food composition, and the relationships among absolute quantities—such as the rates of nutrient intake, retention and excretion—cannot readily be extracted from percentage data.

An alternative approach, first discussed in the context of motivational theory (McFarland & Sibly, 1972), is to represent foods as linear trajectories from the origin into n -dimensional state space, where each dimension represents a property or component of the food (e.g. a nutrient). When feeding, the nutritional 'state' of an animal is viewed as moving through the state space in a trajectory coincident with that representing the composition of the current food. In this way, both the proportional composition of a food can be represented (as the angle in state space of the trajectory representing the food), as well as the amounts of the various nutrients ingested (represented as the distance along this trajectory that the state of the animal moves). While the state space models of McFarland & Sibly avoid the major limitation of mixture theory, they have not been widely adopted by researchers interested in nutritional regulation, probably because of their emphasis on animal psychology, and particularly the difficult concept of 'motivation' (Kennedy, 1992).

Over the past few years we have developed the state space approach into an explicitly behavioural and physiological framework, and applied it to the study of insects (Raubenheimer & Simpson, 1993, 1994, 1995, 1996; Simpson & Raubenheimer, 1993a, 1995, 1996; Chambers *et al.* 1995; Simpson *et al.* 1995). One motive has been to provide a simple conceptual and experimental framework for reducing to a series of two-dimensional representations the complex dynamics among various nutrients in an animal's diet. From these representations are extracted tractable parameters for quantitative analysis. A second aim is to provide a unified approach for considering the various aspects of nutritional regulation, including behaviour, post-ingestive physiology, sensory physiology and performance consequences.

In the present paper we present a simple overview of our geometrical models of nutrition, and review selectively work which has been performed on insects using this approach. In addition, we explore the general applicability of the geometrical approach by applying the models to a re-analysis of data for nutrition in rats and chickens.

Identifying intake requirements

An important reference point in any attempt to understand physiological, behavioural or evolutionary aspects of nutrition is the animal's nutritional requirements. These are depicted in our models as points in multi-dimensional *nutrient space*, where each dimension (axis)

represents a required nutrient. The point in nutrient space representing the intake requirement is termed the *intake target*. Subrequirements can be described in a similar way; thus, the optimal nutrient requirements at the level of the tissues (i.e. excluding the fraction of ingested nutrient which is inevitably lost in the faeces) is the *nutrient target*, and that required for growth, the *growth target* (Raubenheimer & Simpson, 1993; Simpson & Raubenheimer, 1993a). Throughout the present paper axes are scaled in units of mass, although the approach applies for any biologically meaningful units (Simpson & Raubenheimer, 1993a, 1995).

In addition to nutrient requirements, an animal's current nutritional state (the proportion of requirements ingested) can also be visualized as a point in nutrient space. To minimize any discrepancy between current nutritional state and the intake target, the animal must 'move' through nutrient space, and this it does by regulating the selection and intake of foods. Each food is represented by a linear nutritional *rail* that projects into nutrient space from the origin at an angle which is defined by the balance of the nutrients it contains. Thus, a nutritionally balanced food—i.e. one that contains the required proportion of the various nutrients—passes through the intake target. This is depicted in Fig. 1a, for an animal that requires in its diet a 1 : 1 ratio of two hypothetical nutrients, A and B. When ingesting the balanced food, the current state of the animal moves outwards in nutrient space along a trajectory which is coincident with the 1 : 1 food rail, and its movement is confined to this trajectory. In order to satisfy its nutrient requirements, the animal need only regulate the amount of the 1 : 1 food that it eats.

By contrast, Fig. 1b depicts the situation if the same animal had available only a nutritionally imbalanced food, containing 1 part of nutrient A to 2 parts of nutrient B (food 1 : 2). In this case, the intake target cannot be reached and the animal's behaviour inevitably leads to conflict between the requirements for the two nutrients: in order to ingest enough of A, it would need to ingest an excess of B (point (ii) in the figure), while if it satisfied its optimal requirement for B it would suffer a deficit of A (point (i)). Alternatively, it could settle for some intermediate strategy in which it suffers both a deficit of A and an excess of B (e.g. point (iii)).

The animal could, however, satisfy its optimal nutrient requirements if it had available two nutritionally imbalanced foods, providing the rails representing these fall on opposite sides of the intake target. In this case, the foods are nutritionally *complementary*, and by switching appropriately between them the animal can reach the intake target, or any other point in the triangular section of the nutrient plane which is bounded by the rails (Fig. 1c).

Assuming that under *ad lib.* conditions animals regulate so as to optimize performance, the situation of Fig. 1c provides a powerful experimental means of locating the position of the intake target. The rationale here is that an animal which has accessible (by virtue of the available foods) an area of nutrient space that contains the intake target should regulate to this point. Such experiments are considerably strengthened if two or more treatment groups are included, which have available different combinations of complementary foods (see Simpson & Raubenheimer, 1995). If the same point of nutrient intake is *defended* by animals in all treatment groups, then this confirms that the intake point does in fact represent the outcome of homeostatic regulation for the nutrients in question. Independent confirmation that the defended point corresponds with the intake target can be obtained using performance criteria, as discussed below.

Fig. 2 provides examples of two-dimensional regulation for protein *v.* carbohydrate intake in locusts, rats and broiler chickens, and salt *v.* macronutrient intake in locusts.

It might be, under some circumstances, that in a food switching experiment no such defence of a point in nutrient space is indicated. There are several possible explanations for this, which are discussed in some detail by Simpson & Raubenheimer (1995). Firstly, intake of one or more of the nutrients might not be regulated within the time scale of the experiment.

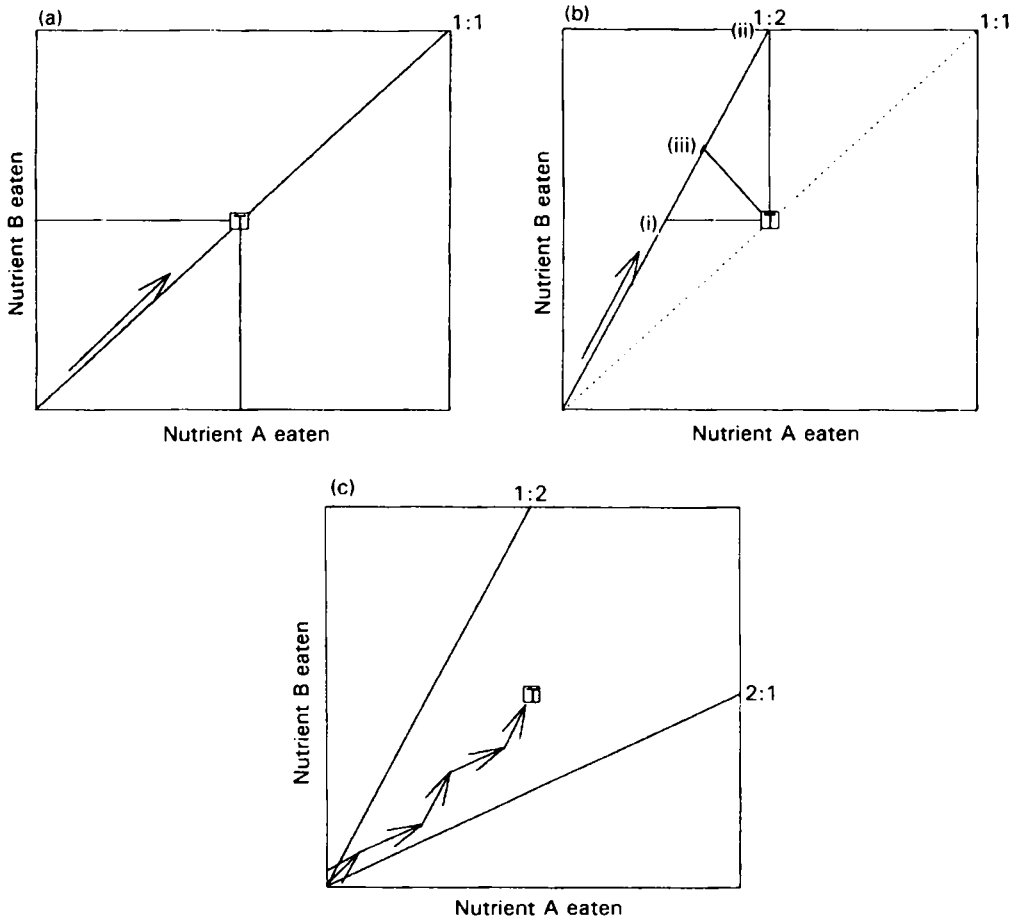


Fig. 1. Schematic illustration of nutrient regulation in two dimensions, one for each of nutrients A and B. The boxed 'T' represents the intake target of a hypothetical animal (i.e. the optimal balance and amounts of the nutrients required). Foods are represented by linear trajectories from the origin into nutrient space (nutritional rails), whose angle relative to the axes is determined by the balance of the nutrients A and B they contain. Thus, in (a), the food contains a 1 : 1 ratio of the nutrients (indicated by the two-digit figure at the distal end of each rail). This is the same ratio of the nutrients optimally required by the animal (i.e. the 1 : 1 food is nutritionally balanced), and by feeding on this food the animal can thus reach the intake target. In (b), however, the animal has access only to food 1 : 2, and can therefore not reach the intake target, but must adopt a compromise between overingesting nutrient B and underingesting nutrient A (points (i), (ii) and (iii) represent possible points of compromise). In (c), the animal has access to two nutritionally imbalanced, but complementary foods. By switching appropriately between them, it can move through nutrient space to the intake target. Any biologically meaningful units might be used to scale the axes.

Secondly, the nutrients might have been presented in a manner which results in pathological responses—for example the nutrient sources are so extreme in composition as not to be recognized as food. Thirdly, the axes might have been incorrectly or incompletely defined. Some nutrients, like proteins, are a mixture of several components (amino acids), and are thus

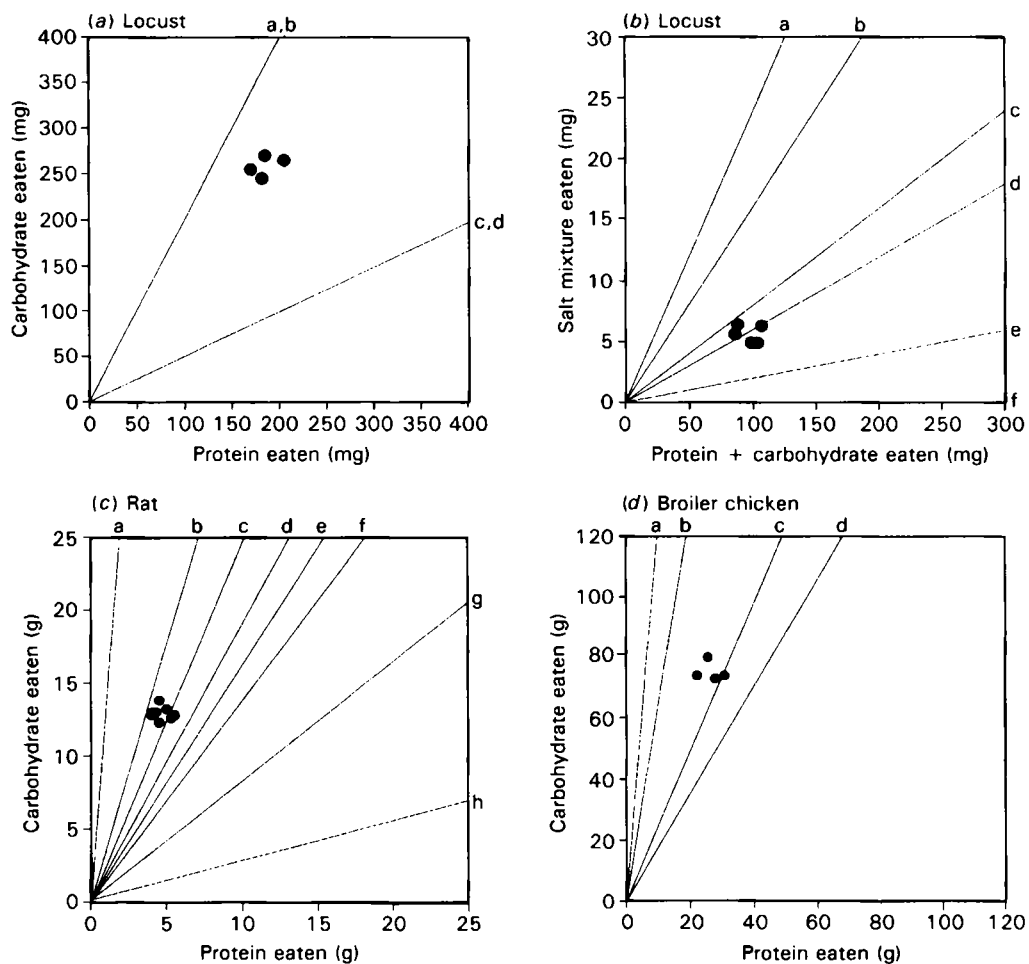


Fig. 2. Mean results from experiments indicating defence of a point of intake in the face of variation in nutrient composition of two choice foods. (a) Data from locusts fed over 6 days of the fifth larval stadium on one of four pairings of synthetic foods, containing either 14:28 or 7:14%protein: %digestible carbohydrate (rail a,b) with either 28:14 or 14:7 (c,d). Foods contained identical concentrations of other nutrients, totalling 4%, and the remainder was indigestible cellulose. Data are from Chambers *et al.* (1995). (b) Locusts fed synthetic foods containing a fixed concentration of protein and digestible carbohydrate (totalling 30%) and one of 7 concentrations of salt mixture over the first 2 days of the fifth stadium. Food pairings were a v. f, b v. f, c v. f, c v. e, d v. e. Data are from Trumper & Simpson (1993). (c) Results from adult male rats provided with pairs of foods varying in protein and carbohydrate content. Pairings were a v. e, a v. g, a v. h, b v. c, b v. d, b v. f, b v. g, b v. h. Data are from Theall *et al.* (1984), reinterpreted by Simpson & Raubenheimer (1997). (d) Defence of protein-carbohydrate intake by broiler chickens provided with one of four food pairings, a v. c, b v. c, c v. d, b v. d. Data come from Shariatmadari & Forbes (1993) with information on dietary carbohydrate and lipid composition from J.M. Forbes (pers. comm.).

represented by *compound axes*. A compound axis should not be included in a model unless it approximates to a nutritionally balanced mixture of components.

Rules of compromise: when the intake target cannot be reached

Measuring rules of compromise

Animals might in many circumstances have access only to foods which are to some extent nutritionally imbalanced, and which thus do not enable them to reach the intake target (as depicted schematically in Fig. 1*b*). An animal in this predicament must arrive at a suitable compromise between overingesting some of the nutrients and underingesting others (see Raubenheimer (1992) for further discussion on such ingestive trade-offs). The regulated point of intake in this circumstance—the *rule of compromise*—should reflect the relative costs and benefits of overingesting one of the nutrients and underingesting the other.

Having determined the position of the intake target, the rule of compromise can be measured simply by confining animals to a single, nutritionally imbalanced food and measuring their intake of the nutrients over the relevant period. In such an experiment, the rule of compromise can be described as the relationship between the position in nutrient space of the regulated intake point and the intake target. Thus in Fig. 1*b*, animals reaching point (i) regulate intake to the target coordinate for nutrient B, but suffer a deficit of A; those at point (ii) regulate exclusively for nutrient A, and those at point (iii) regulate to an intermediate point.

However, the results of such an experiment including only one imbalanced food are limited, since there is no guarantee that animals would respond in the same way to foods which are more, or less, imbalanced with respect to nutrients A and B, or indeed to those which are imbalanced in the opposite direction (e.g. food 2:1 from Fig. 1*c*). Rules of compromise should thus be measured for several imbalanced foods, whose rails cover a range of angles in nutrient space. Such an experiment would give rise to an array of intake points, and the shape of this *intake array* provides a comprehensive description of the rule of compromise for the nutrients under consideration (the practicalities of such experiments have been discussed in detail by Simpson & Raubenheimer (1995)). Figs 3*a–c* show some hypothetical intake arrays; further possibilities together with their interpretation are presented in Raubenheimer & Simpson (1993).

Interpreting rules of compromise

Intake arrays thus provide a visual representation of the patterns of nutrient intake when animals trade-off over- and under-ingesting nutrients in nutritionally imbalanced foods. To interpret what the various intake arrays mean in terms of the over- and under-ingestion of nutrients, it is useful to produce further summary plots of the data.

Figs 4*a–c* show an example of such summary plots, produced for the data presented in Figs 3*a–c* respectively. Plotted on the x-axis in these summary plots is the rail angle (in radians) and on the y-axis is a measure of the nutritional error (distance from the A and B coordinates of the intake target) experienced by animals fed the various foods. Thus each rail has potentially associated with it two errors, one for nutrient A (measured on the x-axis in Fig. 3) and one for nutrient B (y axis in Fig. 3). The horizontal line at zero error represents the target position (no

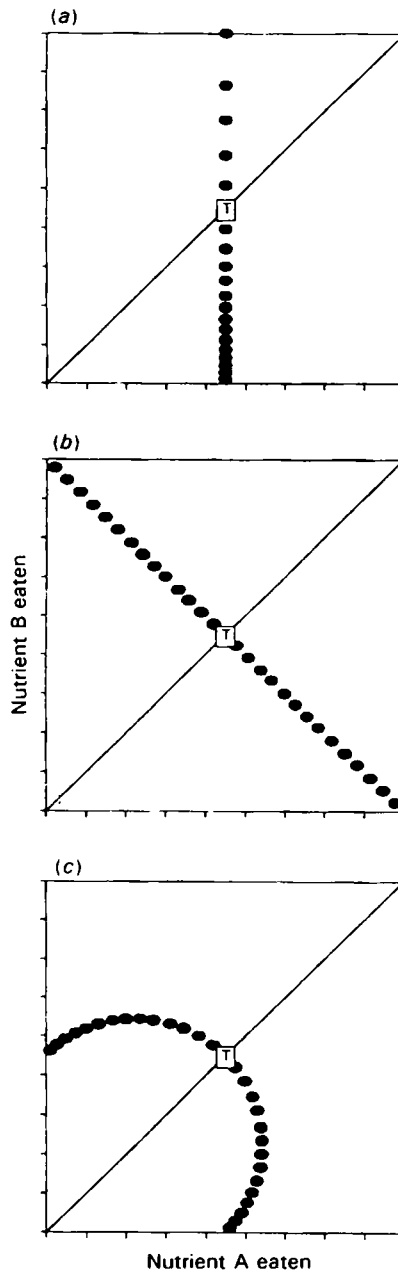


Fig. 3. Hypothetical outcomes from experiments in which animals are fed one of a number of foods differing in the balance of two nutrients, A and B. The boxed 'T' represents the intake target, and the filled circles represent the intake of the two nutrients by animals fed foods which were nutritionally imbalanced and thus did not enable them to reach the target. The shapes of such intake arrays represent the rules of compromise which animals adopt when feeding on nutritionally imbalanced foods. (a) *No interaction rule*; (b) *Equal distance rule*; (c) *Closest distance rule*. See text and Figs 4 and 5 for interpretation of these rules. For discussion of the appropriate units of scaling for axes see Simpson & Raubenheimer (1993a, 1995).

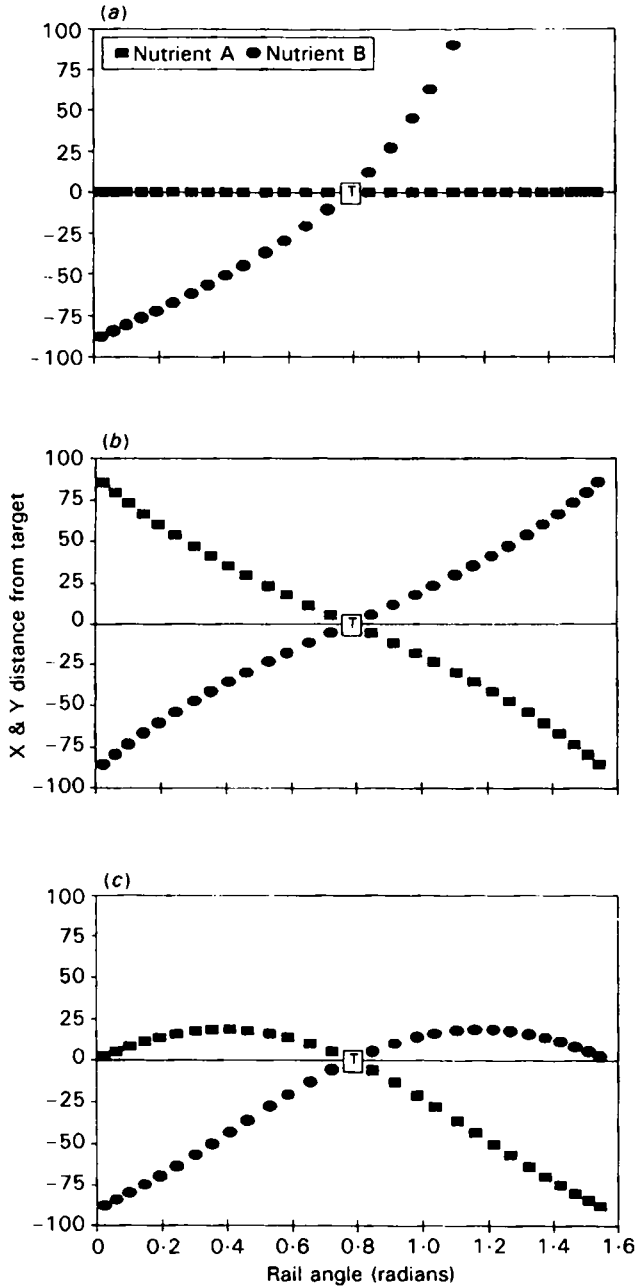


Fig. 4. Relationship of the over- and under-ingestion of nutrients A and B with rail angle (nutrient imbalance) for hypothetical animals adopting the rules of compromise *no interaction* (a), *equal distance* (b) and *closest distance* (c). The horizontal line represents zero error for both nutrients, which is only achieved by animals fed the target food (indicated by the boxed 'T'). Positive values represent an excess of either nutrient, and negative errors a deficit. The ordinate is scaled using the same units as Fig. 3.

error for either nutrient), while a positive error represents an excess and a negative error a shortfall of a nutrient.

It is easy to see in these terms what the array in Fig. 3a means: here the animals have regulated intake so as to satisfy without error their requirements for nutrient A, irrespective of the excess or deficit incurred for nutrient B (Fig. 4a). We will refer to this as the *no interaction* (NI) rule. By contrast, the equivalent plot for the array in Fig. 3b shows that the rule of compromise displayed here is to regulate intake such that the deficit incurred in the one nutrient exactly balances the excess incurred in the other (Fig. 4b); this is, accordingly, termed the *equal distance* (ED) rule.

It is not, however, as easy to interpret in these terms what the intake array in Fig. 3c means. This requires another summary graph, which plots against rail angle the sum of the absolute value of the two errors (error in A + error in B) incurred by animals fed each food (Fig. 5a; note that the sum of errors is equivalent to the distance between the two data points corresponding with each rail in Fig. 4). This Fig. shows that the sum of the errors for the two nutrients is lower for the intake array in Fig. 3c than for the ED rule; in fact, the former represents the minimum

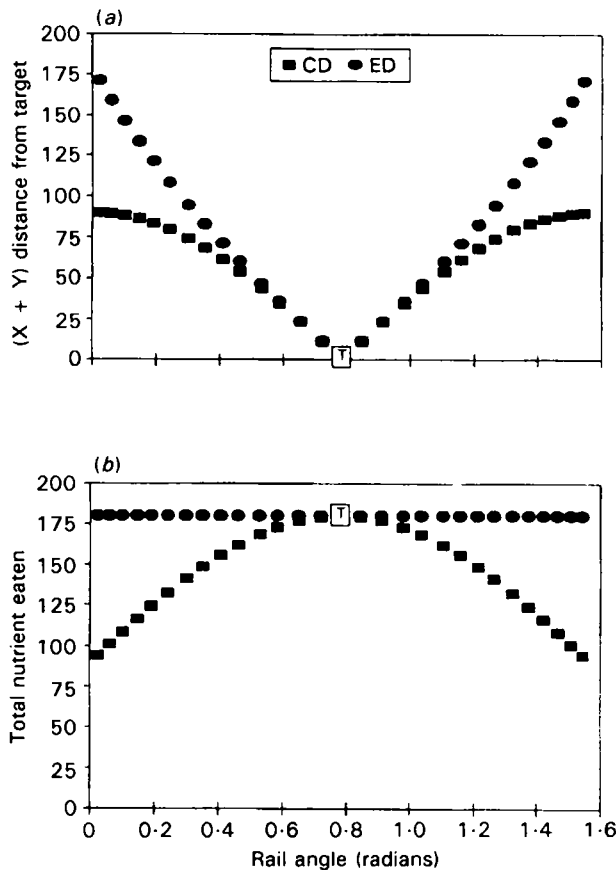


Fig. 5. (a) Total distance (i.e. the sum of the x and y distances in Fig. 3) from the intake target (boxed 'T'), as a function of nutrient imbalance (rail angle), suffered by hypothetical animals adopting the rules of compromise *closest distance* (CD) and *equal distance* (ED). (b) The sum of the amounts of nutrient A and B eaten by the same animals. The ordinate is scaled using the same units as Fig. 3.

possible two-dimensional distance that can be achieved from the intake target. This rule therefore corresponds with feeding to the point in nutrient space which, for each imbalanced food, achieves the closest possible geometrical (in this case two-dimensional) distance from the intake target. We have, accordingly, termed it the *closest distance* (CD) rule (Raubenheimer & Simpson, 1993; Simpson & Raubenheimer, 1993a).

A particularly interesting comparison, to which we shall return below, is that between the patterns of regulation of nutritional error in the ED (Fig. 4b) and the CD (Fig. 4c) rules. It can be seen from this comparison that animals adopting the ED rule ingest greater amounts of the excess nutrient in the food than those adopting the CD rule. Accordingly, the total nutrient gain is greater for ED than for CD but, as mentioned above, the summed nutritional error is smaller for CD (Fig. 5a). A direct comparison of total nutrient gain under the two strategies is presented in Fig. 5b. Note that ED corresponds with defending a constant nutrient gain, irrespective of rail. For CD, by contrast, it is only animals on the target rail which gain as much nutrient as those adopting ED, with total macronutrient gain decreasing progressively with increasing dietary imbalance.

Examples of intake arrays

D. Raubenheimer & S. J. Simpson (1993 and in preparation) compared the rules of compromise for protein and carbohydrate in two species of locust, the African migratory locust (*Locusta migratoria*) and the desert locust (*Schistocerca gregaria*). The migratory locust is a nutritional specialist, feeding only on grasses, while the desert locust is a relative generalist, feeding on grasses as well as a wide range of dicotyledonous plants. It might be expected that generalists such as the desert locust would be more capable of capitalizing on excess available nutrients in imbalanced foods, even if this meant deviating further in nutrient space from the intake target; it should thus tend toward the ED rule (Fig. 5). By contrast the specialist migratory locust should be more sensitive to the magnitude of deviations from the intake target, and should thus adopt the CD rule. Fig. 6 shows that the data supported this expectation: over the first three days of the final larval stadium the intake array for the desert locust fitted the ED model, while that for the migratory locust was consistent with the CD rule. An interesting exception is the intake point for migratory locust feeding on the food containing 7% of carbohydrate and 35% of protein (the point closest to the carbohydrate axis), where a greater excess of protein was tolerated than predicted by the model. A probable reason for this deviation from the CD rule is that locusts are capable of deaminating proteins and channelling the carbon skeletons into energy metabolism (Zanotto *et al.* 1993, 1994). This would not only provide a means for eliminating some of the ingested excess of proteins, but also for partly redressing the carbohydrate deficit incurred on this extreme food.

Rules of compromise under different circumstances are presented in Fig. 7. The first graph in the panel shows the rule for the African migratory locust when trading-off the intake of salt against a balanced complement of all other nutrients, which resembled very closely the NI rule depicted in Fig. 3a. Note that this outcome does not reflect a general failure to regulate the intake of salt, since in a food switching treatment the intake of salt was regulated tightly (Fig. 2b). Rather, these insects conditionally abandon the regulation of salt intake in circumstances where this conflicts with macronutrient requirements. A pattern resembling the NI rule was also shown for protein and carbohydrate regulation by adult male rats provided with foods containing a lower than optimal protein:carbohydrate ratio (Fig. 7b). Here the rats abandoned carbohydrate regulation to maintain constant intake of protein. However, when young male rats

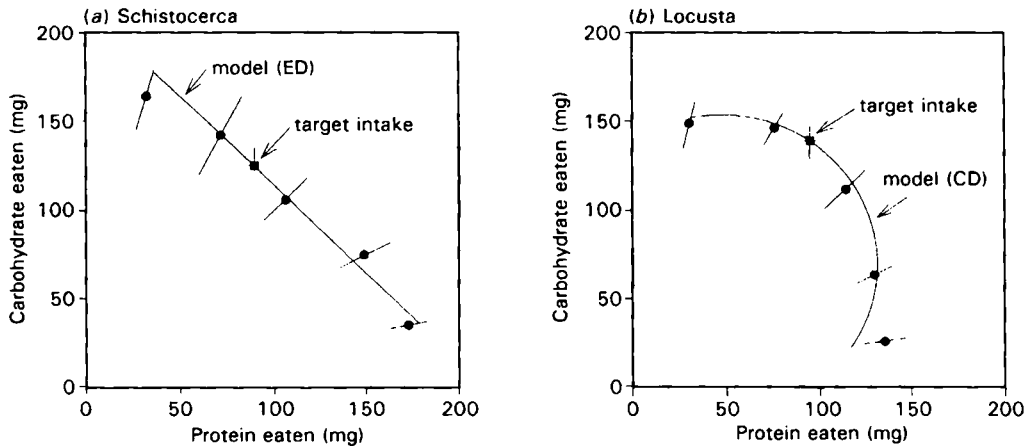


Fig. 6. Protein-carbohydrate intake arrays for (a) the desert locust (*Schistocerca gregaria*) and (b) the African migratory locust (*Locusta migratoria*) over the first 3 days of the fifth larval stadium. Filled circles represent mean (\pm SE) intake of 10 insects fed synthetic foods containing the nutrients in a balance of (%protein : %digestible carbohydrate) 7 : 35, 14 : 28, 21 : 21, 28 : 14 or 35 : 7 (from left to right in the figure). The solid square represents the selected point of intake by locusts allowed to switch between food 14 : 28 and 28 : 14 (target intake), while the solid line represents the prediction for the *equal distance* (a) or *closest distance* (b) rules of compromise. Diagonal standard errors are plotted for the no-choice treatments since for these the levels of intake of the two nutrients were deterministically linked. In the switching treatment, the intake of the nutrients could be regulated separately and individual standard errors are therefore plotted. Data from D. Raubenheimer & S.J. Simpson, in preparation.

which were protein loaded prior to the experiment were provided with foods containing higher than optimal protein : carbohydrate ratios they tolerated a higher intake of protein than expected under the CD model, thereby gaining extra limiting carbohydrate (Fig. 7c). Layer chickens on suboptimal foods balanced intake of protein and carbohydrate such that errors in protein intake relative to the intake target were smaller than those tolerated for carbohydrate (Fig. 7d).

Changes across physiological, developmental and evolutionary time

Intake targets and nutritional rules of compromise have been considered thus far as patterns integrated over a fixed time period. In fact, both targets and intake arrays are trajectories which move over several time scales.

Data exist for the movement of intake targets over three time scales. First, the movement of targets in physiological time in response to an animal's environment and recent nutritional and ecological history has been measured. For example, Fig. 8a shows that the selected point in a protein-carbohydrate nutrient space shifts towards increased carbohydrate intake in recently flown locusts compared to unflown controls; other examples for insects include Simpson *et al.* (1991), Simmonds *et al.* (1992) and Chambers *et al.* (1995). Similarly, Fig. 8b shows the effect of environmental temperature on protein and carbohydrate intake in weanling male rats. Secondly, targets move in developmental time as the animal grows, reproduces, senesces and responds to its longer term nutritional experience (Fig. 9 shows examples for both insects and rats). Thirdly, they move in evolutionary time as animals are selected (either naturally or

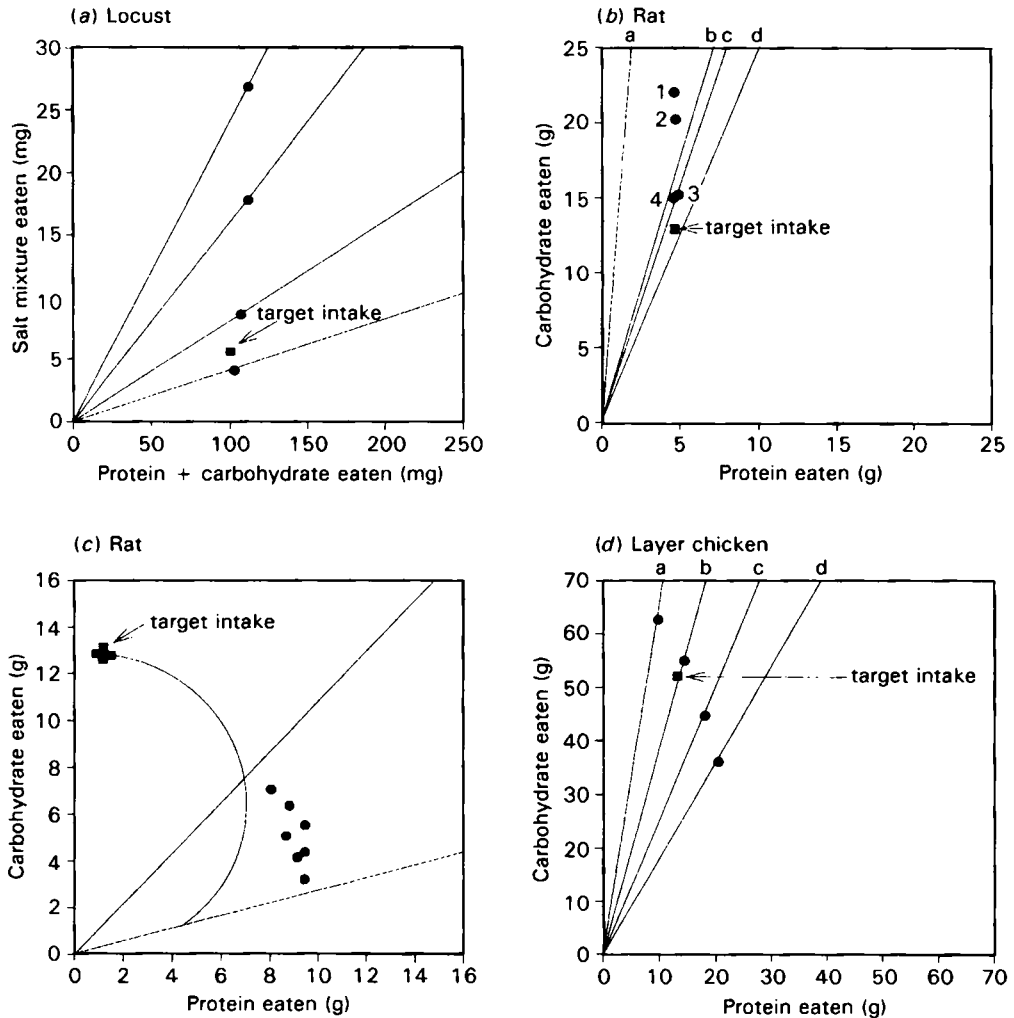


Fig. 7. Responses in animals fed foods which do not allow the intake target to be reached. (a) Locusts provided with foods containing suboptimal salt levels (see Fig. 2b), from Trumper & Simpson (1993). (b) Data from Theall *et al.* (1984), as reinterpreted by Simpson & Raubenheimer (1997). Adult male rats were given food pairings a v. b (point 1), a v. c (point 2), b v. c (point 3) or b v. d (point 4). The defended target intake for protein v. carbohydrate is shown as a square (see Fig. 2c). Note how the rule of compromise under circumstances where animals could not achieve the target intake, because both foods contained a lower than target protein : carbohydrate ratio, was to abandon carbohydrate regulation while strongly defending protein intake. (c) Data from Tews *et al.* (1992), for young, protein loaded male rats which were either allowed to choose between foods which encompassed the target intake (5 treatments converge on the same intake point in the cluster of circles at the top left), or else were provided with one of 7 treatments where the two foods both contained higher than the target protein : carbohydrate ratio (the pairings fell within the dotted rails). In these cases rats weighted overeating protein less strongly than undereating carbohydrate, relative to the *closest distance* rule (shown by the arc; see text). From Simpson & Raubenheimer (1997). (d) Results recast from Shariatmadari & Forbes (1993) for layer chickens given either a single food (rails a–d) or a choice of a v. d. Diet b allowed chickens to reach their target. When they were unable to reach the target intake they weighted protein regulation more strongly than that for carbohydrate, relative to the *closest distance* rule (where birds would have moved to the point on their food rail which lay at right angles to the target).

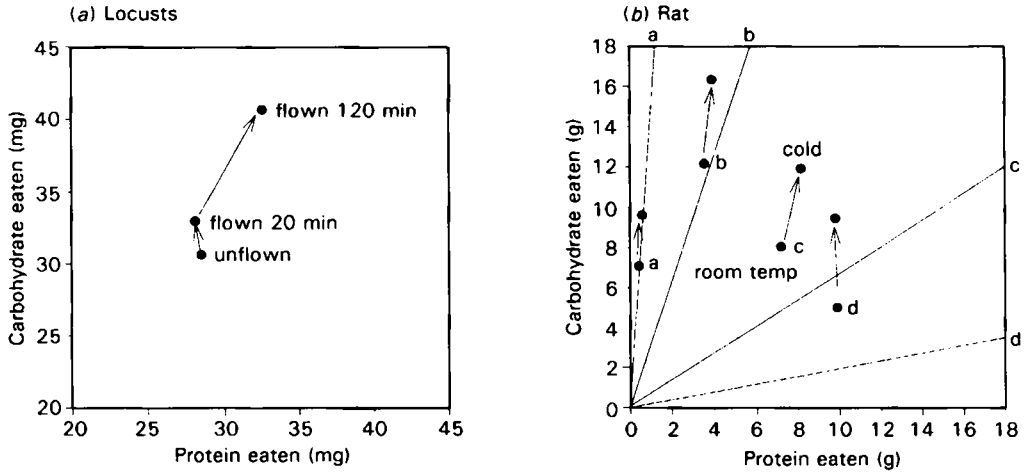


Fig. 8. Movement of intake targets in physiological time. (a) The effect of flying on subsequent nutrient selection by adult female *Locusta migratoria* given foods 14 : 28 and 28 : 14 (%protein : %digestible carbohydrate). Flying resulted in a shift in the selected intake point predominantly towards increased carbohydrate intake (D. Raubenheimer & L. Firth, in preparation). (b) The effect of environmental temperature on nutrient intake in weanling male rats. Animals were fed a food with no protein and 84% carbohydrate (rail equals the y-axis) and food a, b, c or d (all totalling 84% protein + carbohydrate). While the intake points reached were constrained by the nutrient area available within a treatment, in each case keeping the animals in the cold rather than at room temperature led to increased carbohydrate but not protein consumption. Data from Musten *et al.* (1974) in Simpson & Raubenheimer (1997).

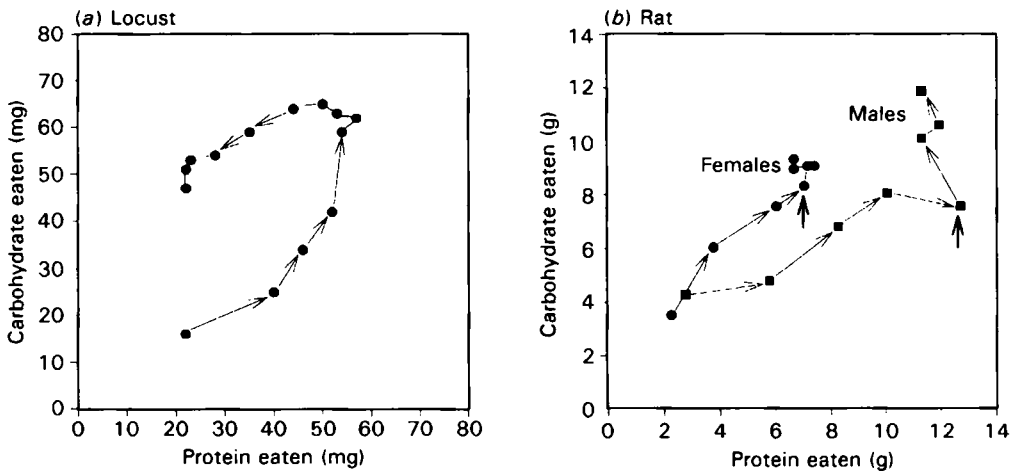


Fig. 9. The movement of intake targets in developmental time. (a) Changes in daily intake of protein and carbohydrate in adult female locusts (*Locusta migratoria*) allowed to select between foods from the time of moulting until the end of the somatic growth phase. Data from Chyb & Simpson (1990). (b) Intake by male and female rats provided with pure macronutrient sources from weaning until maturity. Each point represents the average daily intake over a given week. The heavy arrows indicate the time of puberty. From Simpson & Raubenheimer (1997) after Leibowitz *et al.* (1991).

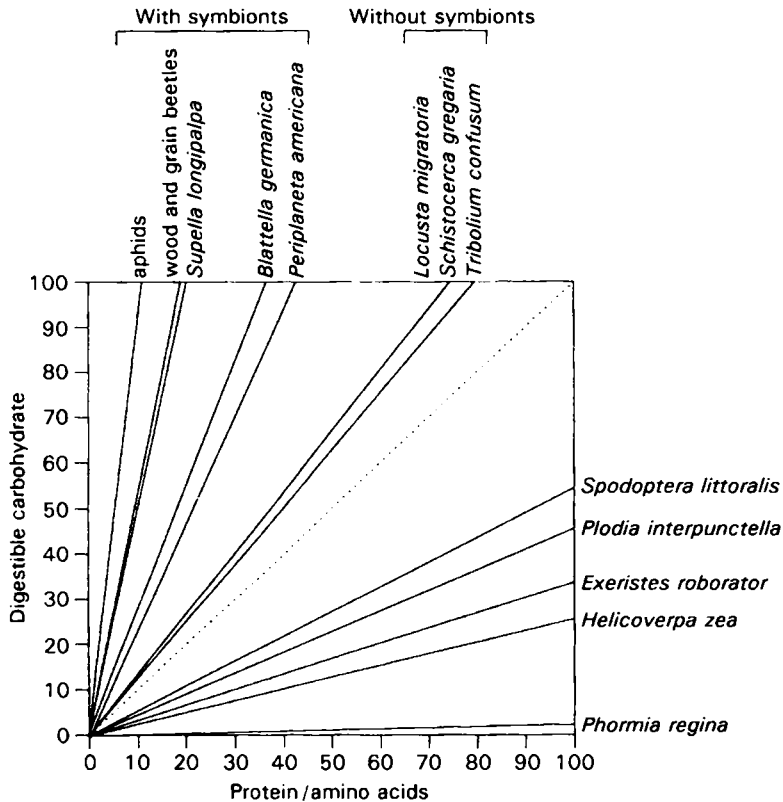


Fig. 10. Selected data from a comparative analysis of the larval requirements of 117 insect species (Simpson & Raubenheimer, 1993a), showing how the optimal dietary protein : carbohydrate ratio varies with life-history strategy and phylogeny. Insects with the lowest protein : carbohydrate requirements are those with endosymbiotic bacteria which contribute to nitrogen metabolism. At the other extreme, high protein : carbohydrate requirements are found mainly in insects which acquire the majority of their adult protein needs as larvae.

artificially) to utilize different sources of nutrient and evolve differing life-history strategies (Fig. 10).

By contrast, with intake targets there are relatively few data on the changes over time in rules of compromise. For developmental time, D. Raubenheimer & S.J. Simpson (1993 and in preparation) measured the changes in the intake array for protein and carbohydrate in the desert locust and the African migratory locust over the first five days of the final larval stadium. As shown in Fig. 6, the array for the former species over the first three days resembled the ED rule, while that for the latter species resembled more closely the CD model. By the fifth day of the stadium the shape of the arrays for both species had not changed appreciably from that on day 3 (Fig. 11). These data further support the prediction above that the ED rule would be the expected strategy for a generalist species, and the CD rule for a specialist.

The only other data available for changes with time in nutritional rules of compromise are from a comparative analysis of a range of insect species (Simpson & Raubenheimer, 1993a). Interestingly, the data suggested that for protein and carbohydrate balance, the CD rule is a strongly conserved strategy among insects: in addition to the migratory locust, caterpillars,

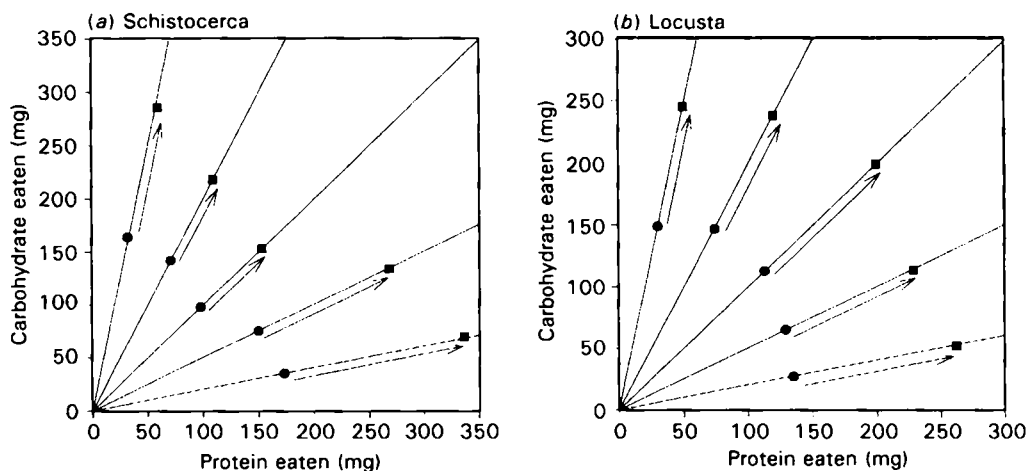


Fig. 11. Movement of protein : carbohydrate intake arrays from the third (inner array) to the fifth day into the fifth larval stadium of *Schistocerca gregaria* (a) and *Locusta migratoria* (b). Details are as in Fig. 6.

larval beetles, aphids and adult and larval cockroaches all appear to adopt this strategy (Abisgold *et al.* 1994; Simpson & Raubenheimer, 1993a).

Mechanisms of regulating intake

Defending a point of nutrient intake in the face of foods varying in composition involves two general categories of component mechanisms: innate responses to sensory properties which correlate with a food's nutrient composition, and modulatory nutrient feedbacks which enable an animal to compensate for short term environmental vicissitudes (Simpson & Raubenheimer, 1993b). The most commonly employed sensory cue is taste, with nutrients such as sugars and amino acids acting as phagostimulants for animals of most taxa.

Innate regulatory components

We have recently developed a class of models which describe some fundamental aspects of the innate properties of the nutritional regulatory systems of both vertebrate and invertebrate animals (Simpson, 1994; Simpson & Raubenheimer, 1996). If an animal is provided with two foods varying in their content of a nutrient, A, with one food having more than the optimal level of A and the other less, then the animal can mix an optimal diet by distributing its consumption between the two foods as follows:

$$p_1 \cdot e + p_2 \cdot (1 - e) = p_t \quad (1)$$

where p_1 , p_2 and p_t are the proportions of A in foods 1, 2 and the optimal food, respectively, and e and $(1 - e)$ are the proportions of total intake from foods 1 and 2 respectively. The design problem facing the animal is to solve this equation for e . This can be achieved if the relative phagostimulatory powers of two or more foods (their ability to initiate and maintain feeding) are a direct function of the relative amounts which need to be ingested to reach the target level

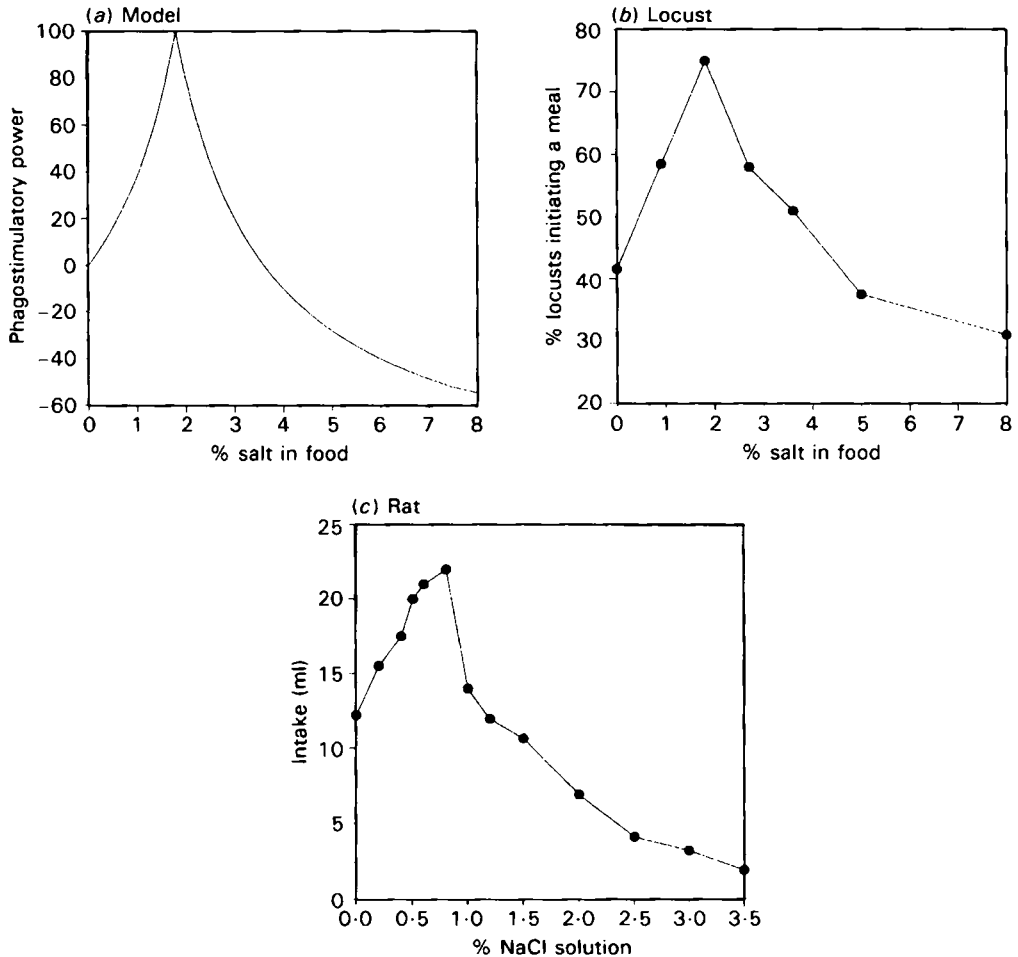


Fig. 12. (a) Theoretical prediction for taste responses to dietary salt in locusts based on the model in equation 2 (see text) and the target intake of 1.8% salt mixture in the diet reported by Trumper & Simpson (1993; see Fig. 2b). (b) Data supporting the model's predictions from Simpson (1994), showing the probability of locusts initiating feeding on first contact with synthetic foods varying in salt content. (c) Intake of saline over 60 min by rats (Weiner & Stellar, 1951). Although it is not possible to equate the x-axes directly, the relationship has a closely similar shape to the model in (a), which on theoretical grounds would be expected to apply to any taste system mediating nutritional homeostasis in an environment where sources of nutrient occur at both lower and higher than optimal concentrations. From Simpson & Raubenheimer (1996).

of nutrient intake. Hence, the phagostimulatory power of a given food (P_x) should vary with its concentration of A, reaching a maximum at the optimal concentration and declining steeply at lower and higher levels. For the simple case of regulation of one nutrient, A, this relationship is stated as:

$$P_x = \left(\frac{pt}{pt + |pt - px|} \right) - 0.5 \times 200 \quad (2)$$

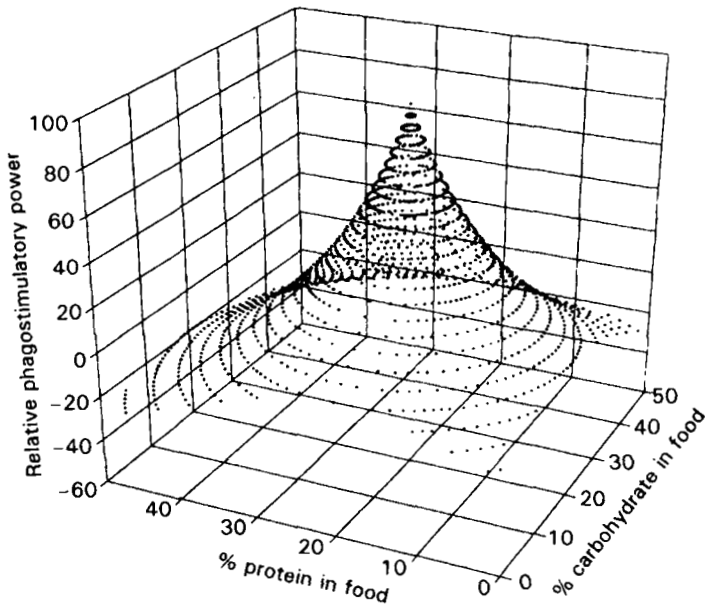


Fig. 13. Expansion of the taste model to include two nutrient dimensions, protein and carbohydrate. The graph plots the predicted phagostimulatory surface for locusts tested with foods varying in protein and carbohydrate content (after Simpson & Raubenheimer, 1996).

where p_t and p_x are the proportions of A in the optimal food and food x respectively. Subtracting 0.5 and multiplying by 200 serve to scale phagostimulatory power such that the optimal food has a phagostimulatory power of 100 and a food with 0% A has a phagostimulatory power of 0. This scaling is arbitrary and yields negative values, which simply mean that such foods are less phagostimulatory than one containing no A.

By distributing the amounts eaten of two (or more) foods according to their relative phagostimulatory powers as given by equation 2, an animal will regulate its consumption of A to the optimal level. If it happens that the available foods are not nutritionally complementary (enclose the optimal level), then most is eaten of the food which is closest to optimal in composition, a fact which is well known from various animals, including locusts (Trumper & Simpson, 1993; Chambers *et al.* 1997), rats (Theall *et al.* 1984) and chickens (Shariatmadari & Forbes, 1993). Similarly, if one of two foods is nutritionally optimal and the other is not, then the degree to which the animal misallocates feeding to the suboptimal food is a defined function of how much it differs from the optimal food (tested and verified for locusts by Chambers *et al.* 1997).

The predictions of this model have been tested experimentally for salt intake in locusts, and are also met when data from rats are reinterpreted (see Fig. 12). The model has also been expanded to include more than one nutrient axis and provides phagostimulatory response surfaces which involve nonadditive interactions between constituent nutrients (Simpson & Raubenheimer, 1996; Fig. 13). Our expectation is that the model will turn out to be widely applicable to taste systems involved in nutritional homeostasis.

Nutritional feedbacks

The above model can provide a mechanism for achieving an optimal concentration of nutrients in the diet, but it cannot on its own provide a means of regulating for the absolute levels of nutrient intake—it can, in other words, assist an animal to locate the target nutritional rail, but not how far along this rail to move (Simpson & Raubenheimer, 1996). Further, since the parameters of such a mechanism are set in evolutionary time, it embodies only information about ancestral environments, and is thus limited in resolution. It cannot, for instance, provide a means to compensate for shorter term quantitative or qualitative perturbations in nutrient availability or requirements. For example, the animal may need to compensate for the effects of nutrient deprivation, or for increases in energy demand due to an unpredictable increase in activity levels or altered thermoregulatory requirements (e.g. Fig. 8). These necessitate short term, nutrient specific feedbacks onto the regulatory mechanisms, acting to modify the phago-stimulatory power of foods (Simpson & Raubenheimer, 1996).

The nature of such short term homeostatic mechanisms in insects has received considerable attention during recent years. While compared with the taste model outlined above the feedbacks involved concern short term information loops (set in physiological and ecological rather than evolutionary time), in actuality there is a range of relevant time scales. At the one extreme are time scales linking current behaviour to an animal's immediate nutritional history, concerning the amounts and balance of nutrients ingested in a previous meal and even in a meal currently being ingested. In insects, such mechanisms include peripheral modulation of taste receptor responses by the levels of blood nutrients, as illustrated by the data presented in Fig. 14 (Abisgold & Simpson, 1988; Simpson *et al.* 1991; Simpson & Simpson, 1992; Simpson & Raubenheimer, 1993*b*). At the other extreme is the nongenetic transfer of information across generations. In locusts and some other insects, information about the developmental environment of adult females is transferred to their offspring *via* epigenetic maternal inheritance (Islam *et al.* 1994; Rossiter, 1996), although the detailed effects on nutritional regulation have yet to be investigated. This phenomenon is functionally equivalent to some categories of information transfer concerning nutritional regulation between adult vertebrates and their offspring, including both *in utero* and postparturition routes of transfer (Provenza & Cincotta, 1993).

Between the within-meal and across-generation extremes are feedbacks due to learning, whereby information obtained from previous experiences during an individual's lifetime is incorporated into homeostatic nutritional responses at a subsequent time. In insects, this includes nonassociative learning (Bernays & Raubenheimer, 1991), aversion learning (Champagne & Bernays, 1991) and learned-specific appetites (Simpson & White, 1990; Trumper & Simpson, 1994; Raubenheimer & Blackshaw, 1994; Raubenheimer & Tucker, 1997). In addition, the developmental path of sensory systems might be determined by the nutritional environment in which an animal develops. Thus, Rogers & Simpson (1997) found that locusts raised in a nutritionally complex environment developed greater numbers of taste receptors on the mouthparts than those raised in simpler, but nonetheless ecologically realistic, environments.

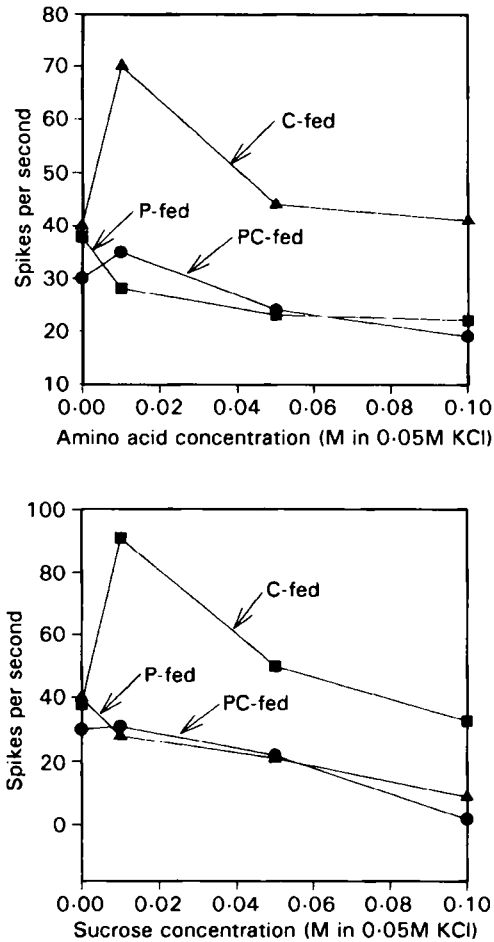


Fig. 14. The effect of nutritional perturbations on the gustatory responsiveness of locusts to sugars and amino acids. Locusts were prefed for 4 h on one of three synthetic foods. The C food contained digestible carbohydrate but no protein; the P food contained protein but no digestible carbohydrate, while the PC food contained both nutrient groups. Data show mean electrophysiological responses from mouthpart taste hairs to stimulation with a range of amino acid and sugar solutions. Note how responsiveness has been modulated by prefeeding, such that receptors respond most vigorously to the nutrients which were absent from the pretreatment food. Such responses have been shown to be mediated directly by levels of amino acids and sugars in the blood, and to be closely associated with changes in dietary selection behaviour. Note also the shape of the dose-response curves, which closely follow the predictions of a two-nutrient expansion of the model in equation 2 (see text and Fig. 13), with nutrient feedbacks providing a qualitative change in nutrient requirements. From Simpson *et al.* (1991) and Simpson & Raubenheimer (1996).

Postingestive regulation

Defence of growth targets

While the levels of the various nutrients ingested in a selected food item are non-orthogonal (i.e. the balance of nutrients is fixed), the balance of nutrients retained can be regulated through selective excretion. In other words, an animal feeding on a food which prevents it from reaching its intake target (e.g. food 1:2 in Fig. 1*b*) can nonetheless reach other nutritional targets, such as the growth target, by altering the efficiency of nutrient utilization. Thus, Raubenheimer & Simpson (1993) performed an experiment in which larval African migratory locusts were fed one of a large number of foods differing in protein and carbohydrate content. To investigate the extent to which growth was defended in the face of such variable nutrient intake we plotted, on the same axes as for intake, the growth derived by each experimental group of insects from protein and carbohydrates. As can be seen in Fig. 15*a*, growth points clustered very tightly around a point in nutrient space, despite the animals in the different experimental groups having varied considerably in the levels of the two nutrients ingested. Similar plots are shown for rats and chickens in Fig. 15*b* and *c* respectively. These data suggest that regulation of the efficiency of utilization of ingested nutrients is an important means of nutritional homeostasis in animals as disparate as insects, mammals and birds.

Quantifying postingestive regulation

Frequently, the efficiency of utilization of ingested nutrients is quantified using ratio based indices—for example, the ratios ADC ($=((\text{consumption} - \text{faeces})/\text{consumption})$) and MEC ($=((\text{consumption} - \text{faeces} - \text{urates})/\text{consumption})$). However, computer simulations have demonstrated that there are serious statistical and interpretative problems with the use of nutritional ratios (Raubenheimer & Simpson, 1992; Raubenheimer, 1995). In their stead, we have recommended that the analysis of covariance (ANCOVA) is used in conjunction with a series of bi-coordinate plots (termed *utilization plots*) which depict the quantitative relationships among the various compartments of nutrient budgets (Raubenheimer & Simpson, 1992, 1994, 1995; Zanutto *et al.* 1993, 1994). Thus, the equivalent of ADC would be investigated for an experimental group of animals by plotting and subjecting to ANCOVA the relationship between nutrient consumed and that retained (consumption – faeces). The relationships among other combinations of nutritional variables (e.g. consumption *v.* growth, consumption *v.* metabolic expenditure, absorption *v.* growth, absorption *v.* metabolic expenditure) may be analysed in a similar way.

Fig. 16*a* is an example of a utilization plot, showing the pattern of post-ingestive homeostatic regulation of protein derived growth in the same insects depicted in Fig. 15*a*. The diagonal dashed line shows the relationship if all of the ingested protein had been converted to growth—in other words, if the efficiency of protein derived growth were complete. The solid line shows the best fit relationship of the observed data. The space between the dashed and the solid lines therefore represents protein which was ingested but lost in the faeces, and thus not converted to growth. Also shown in the Fig. is the protein intake selected when feeding *ad lib.* in food switching experiments as described above (i.e. the protein coordinate of the intake target). The Fig. shows that at levels of protein intake below the optimal point, a large proportion of that ingested was converted to growth. By contrast, animals which were given foods deficient in carbohydrates, and thus made to ingest an excess of protein, showed a low

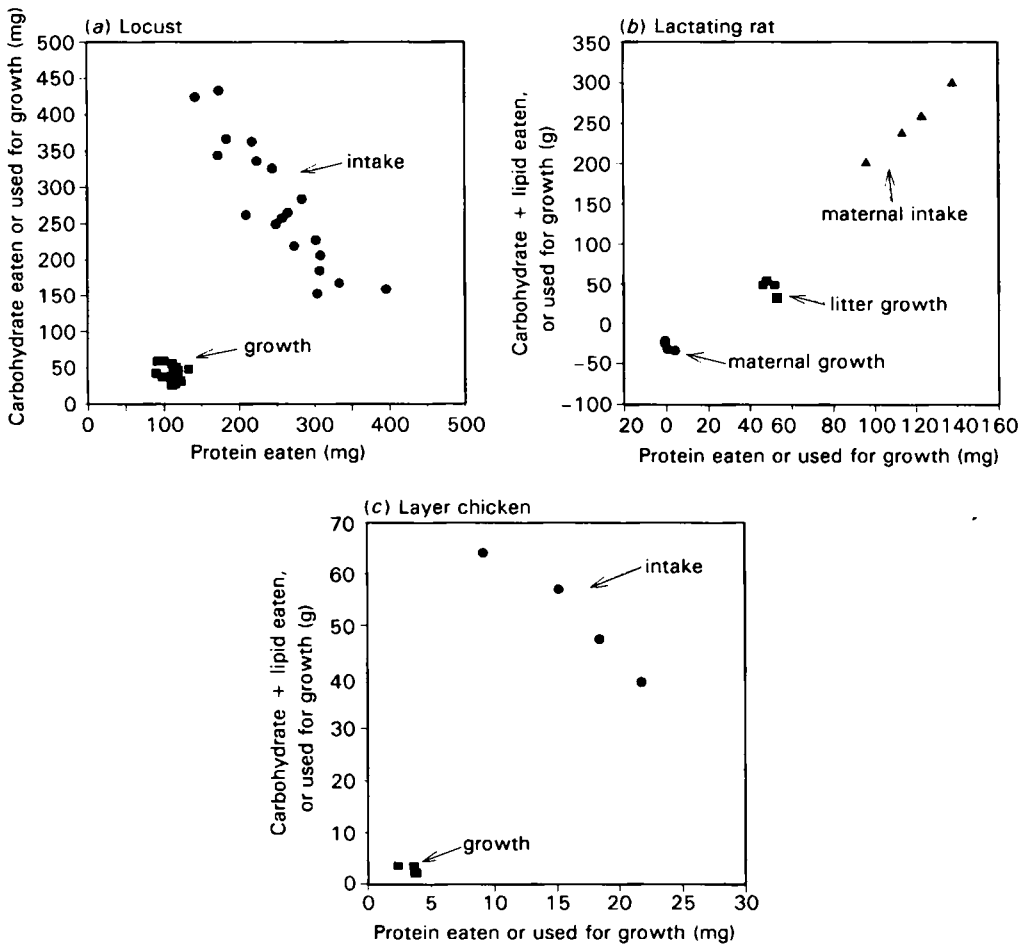


Fig. 15. The relationship between intake and growth, showing how growth is defended in two nutrient dimensions across a range of intakes. (a) Data from Raubenheimer & Simpson (1993) for locusts fed for the entire fifth stadium on one of 19 foods varying in protein and digestible carbohydrate content. The foods contained only trace lipids. Carbohydrate derived growth included lipid and structural carbohydrate in cuticle. (b) Data from Friggens *et al.* (1993) for lactating rats. Maternal intake on the four foods spanned a considerably wider range in both nutrient dimensions than did either maternal or litter growth. The maternal growth target was to maintain body protein content and to lose 20 g of body lipid, while litter growth was regulated at c. 50 g increase in both dimensions. Further analysis of intake data indicated that the pattern of maternal intake represented a rule of best compromise in which protein and energy intake were balanced (not shown). (c) Results recast from Shariatmadari & Forbes' (1993) study on chickens.

efficiency of conversion of protein into growth. Similar analyses have been performed for individual amino acids and carbohydrates in the diet of locusts (Zanotto *et al.* 1993, 1994), as well as for existing data from lactating rats (Fig. 16b) and layer chickens (Fig. 16c).

For locusts, the routes of excretion of protein and carbohydrates ingested in excess of requirements have been investigated. Most of the excess proteins are deaminated and the nitrogen component voided as uric acid in the faeces (Zanotto *et al.* 1993), although small

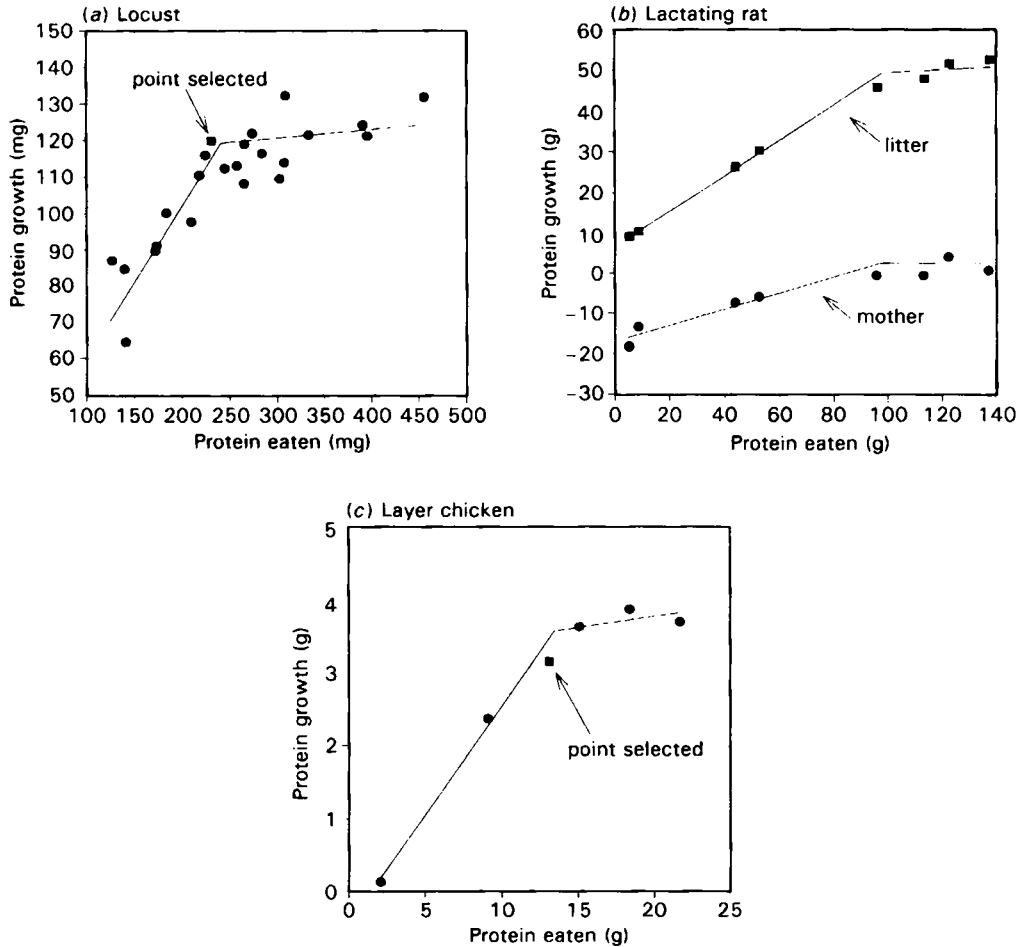


Fig. 16. Utilization plots for protein intake v. protein growth on foods which resulted in a range of protein intakes through variation in protein imbalance relative to other macronutrients. (a) Means for locusts across the fifth larval stadium when fed various foods differing in carbohydrate and protein content. The square symbol indicates protein growth at the defended point of intake (see Fig. 15a) and lies at the transition point in the relationship, as expected if animals regulate intake to the point which optimally meets growth requirements (see text). Data are from Raubenheimer & Simpson (1993). (b) Data from lactating female rats fed foods varying in protein, carbohydrate and lipid content, replotted from Friggens *et al.* (1993). Curves are plotted for both the mother and the litter. Note how in both cases the relationship between intake and growth has a rising phase followed by an asymptote, indicating regulated protein growth (a growth target). In the case of the mother, the target growth was zero and was achieved at c. 100 g protein intake over 12 d. Lower protein intake led to protein loss from the mother to provision the offspring. Litter growth also achieved its plateau (target) level at 100 g maternal protein intake. (c) Data (as g/day) replotted from Shariatmadari & Forbes (1993) for layer strain chickens fed foods varying in protein, carbohydrate and lipid content. Again, a clear two-phase relationship is apparent. The square symbol indicates the protein intake selected by chickens with access to two foods encompassing the intake target (see Fig. 15c). As for locusts, growth achieved at the selected intake is not statistically different from the values for the diets on the asymptote.

amounts of nitrogen are excreted in the form of amino acids (Zanotto *et al.* 1994). Excess carbohydrates, by contrast, do not appear in the faeces but are eliminated as carbon dioxide through an increase in respiratory rate (Zanotto *et al.* 1993, 1997), in a process analogous to diet induced thermogenesis in vertebrates (Armsby & Fries, 1915; Rothwell & Stock, 1979, 1983). More recently, it has been suggested that some vertebrates, too, might use diet induced thermogenesis as a means of nutrient balancing (Trier, 1996). However, Emmans (1987) predicts that constraints on the upper limits of heat loss might restrict the extent to which some homeothermic animals can void excess ingested energy *via* increased metabolic rate.

The interaction of ingestive and postingestive regulation

We have, for practical reasons, considered the regulation of the intake of nutrients and of their subsequent utilization in separate sections. However, postingestive regulation cannot be fully understood without taking into account also the rates and levels of nutrients entering the animal, i.e. without considering also nutrient intake. Raubenheimer & Simpson (1996) therefore developed a modelling approach to conceptualize and quantify the interface between ingestion and utilization, and the relevance of this interface to nutrient balancing.

The model centres on the function relating the transfer of nutrients between serially linked nutritional compartments (*c1* to *c2*) to the time the nutrients have been resident in *c1*, termed a *nutrient transfer function*. Such functions are frequently nonlinear (Sibly, 1981), and this has important consequences for nutritional homeostasis. To illustrate, we will focus on the transfer of a nutrient from the gut of an animal to the blood (Fig. 17*a*). Since the digestion and absorption of the gut contents is a time dependent process, longer gut residence times will yield a higher efficiency of nutrient extraction. However, as constituent nutrients are progressively removed from the gut contents, the rate of nutrient transfer to the blood will inevitably decline, yielding diminishing returns. In order to maximize the rate of nutrient gain in the blood, an animal should void the residual contents of the gut and feed again before the instantaneous rate of transfer drops too far (point *t1* in Fig. 17*a*), but in so doing it loses unprocessed nutrients (denoted 'wastage' in the Fig.). To distinguish the rate 'gain of nutrient per feeding cycle' from the instantaneous rate of gain, we have followed Slansky & Feeny (1977) in adopting from the ecological literature the term '*power*' for the former rate. Animals thus face a trade-off between maximizing power (high throughput) and achieving high nutritional efficiency (longer gut residence time).

These models demonstrate that the periodicity of feeding influences critically the outcome of nutritional regulation, and should thus be subject to strong selection pressure. One important factor which should influence the relationship between ingestive and postingestive nutritional regulation is whether an animal is time limited or nutrient limited: nutrient limited animals should feed at longer intervals in order to maximize the levels of nutrients extracted from foods, while time limited animals should operate at lower efficiency, thereby maximizing power (Raubenheimer & Simpson, 1996).

A second, critically important factor which should influence the quantitative relationship between intake and postingestive efficiency is nutrient balance. Where foods contain a balance of nutrients very different from the requirements of an animal's tissues (as is the case for herbivores), selective retention of the required balance of nutrients might be achieved through the evolutionary determination or the homeostatic regulation of the shape of nutrient transfer functions. This is illustrated in Fig. 17*b* for a hypothetical animal with an intake target of two parts of nutrient B to one part of nutrient A, but feeding on a food containing the nutrients in the

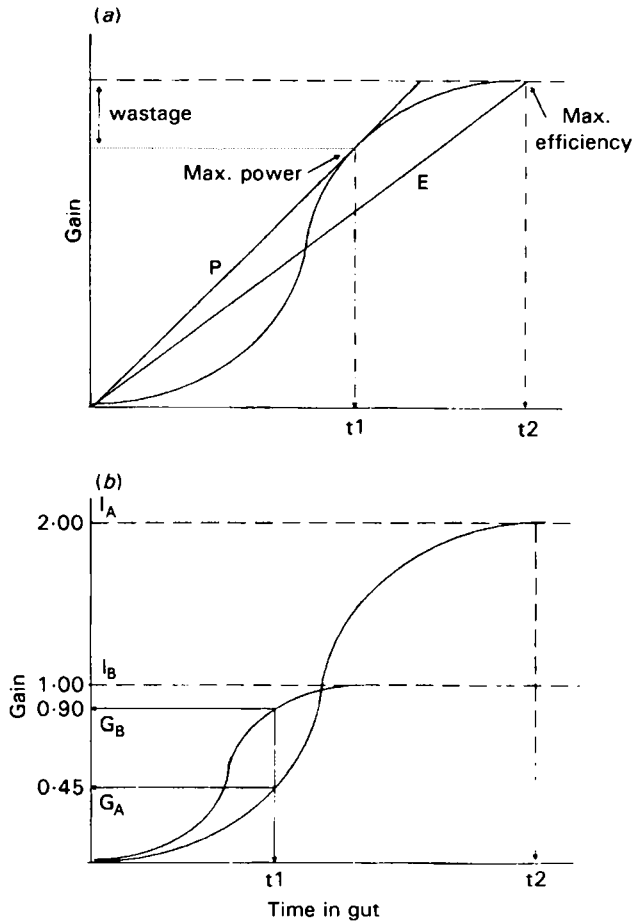


Fig. 17. (a) Transfer function for a single nutrient from the gut to the blood of a hypothetical animal. The origin represents the point at which a batch of food (e.g. a meal) containing a quantity of the nutrient (indicated by the dashed horizontal line) enters the gut. With time the nutrient is progressively transferred to the blood ("Gain"). By voiding the gut contents and feeding at time t_1 the animal achieves the maximum possible rate of gain of the nutrient ('power', the value of which is given by the slope of line P), but loses a quantity of nutrient in the faeces ('wastage') and thus has low efficiency. To maximize efficiency, the animal should feed at time t_2 , but in this case suffers a lower rate of gain indicated by the slope of line E. (b) Transfer functions in a hypothetical animal for two nutrients in an ingested food. The food contains two parts of nutrient A to one part nutrient B, and therefore twice the amount of A is ingested (I_A) than of B (I_B), and this ratio of nutrients would be extracted if the animal fed at time t_2 . However, if required the animal could extract the nutrients in the inverse proportion by feeding at time t_1 , since at this point twice as much of B has been extracted (G_B) than of A (G_A).

reverse proportion. It can nonetheless gain the required balance of nutrients by voiding the gut contents and feeding at a point where the instantaneous gain of nutrient B is twice that of nutrient A (time t_1 in the Fig.).

Fig. 18 shows the nutrient transfer functions for digestible carbohydrate and protein across a single feeding cycle in locusts fed synthetic foods containing 27.5% of each nutrient.

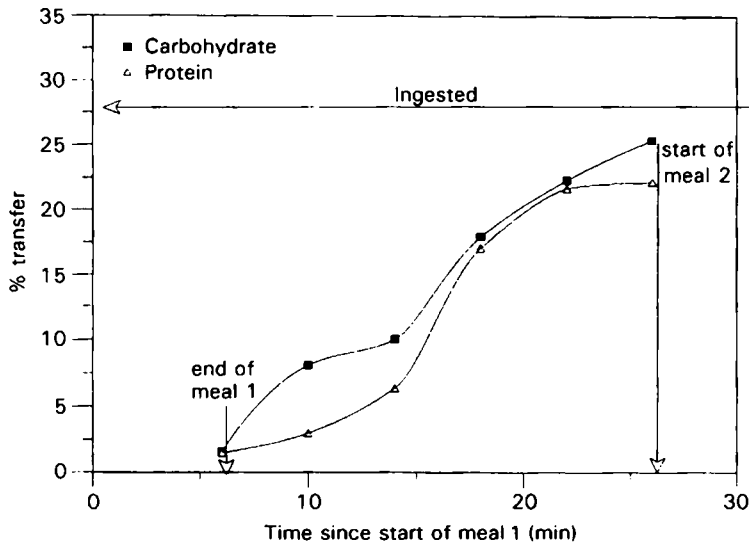


Fig. 18. Nutrient transfer functions for protein and carbohydrate in two-day old fifth instar *Locusta migratoria* fed synthetic foods containing 27.5% of each nutrient. The amount ingested of each nutrient is indicated by the horizontal arrow, and the curves show the rate of removal from the gut to the blood across a single feeding cycle. See text for further discussion. Data from G. Brown, unpublished.

Interestingly, these insects initiate feeding at a point where the ratio of carbohydrate : protein absorbed into the blood is 1.14, which is very similar to the ratio of these nutrients selected in food switching experiments (1.22; Chambers *et al.* 1995).

Performance

To this point, we have discussed the ingestive and postingestive adaptations of animals for achieving nutritional homeostasis, and have presented a family of geometric models for investigating these aspects of nutrition. Both ingestive and postingestive mechanisms, as well as their interaction, exist to subserve a larger outcome: the performance criteria set, in the case of naturally selected animals, by the ecological environment, and for artificially bred (e.g. farm) animals, by the requirements of the breeder. Our models incorporate performance criteria using an additional axis, the *performance axis*, which maps on to nutrient space the consequences for an animal of being at a given point at a given time. Such consequences can be enumerated in various terms, including measures which are obviously related to fitness, such as mortality, development rate, and reproductive output, and less obvious ones, such as activity levels, which in insects influence the probability of death through predation (Bernays, 1997).

A particularly interesting question is the extent to which optima in various performance measures concur with the output of free-running homeostatic mechanisms—in other words, by which measures do animals benefit from the points in nutrient space that they select under *ad lib.* conditions? Presumably, evolutionary fitness is constituted by some compound function of all the relevant variables, and a systematic assessment of such variables provides a means of addressing the functional question of why animals regulate in the ways that they do. It might, of

course, be that maximum performance by different measures corresponds with different intake points in nutrient space, in which case the selected intake point constitutes a trade-off among two or more performance variables.

Examples of performance variables for African migratory locusts are presented in Fig. 19. Locusts fed foods containing a large excess of carbohydrates relative to protein have a low probability of surviving through the fifth larval stadium, as do those fed diets imbalanced in the opposite direction (Fig. 19*a*). The probability of survival increases from both directions towards the more central rails, peaking at the rail estimated using food switching experiments (as described above) to be the intake target. By contrast, two other performance measures are monotonic functions of nutrient balance, but with opposite signs: development rate is highest for locusts fed foods with a large excess of protein (Fig. 19*b*), while the resistance to starvation as adults is highest for those fed during the fifth larval stadium foods containing a relative excess of carbohydrates (Fig. 19*c*). Overall, these measures suggest that nutrient selection behaviour by migratory locusts maximizes the probability of surviving through larval development to reproductive maturity. However, this entails a trade-off with both development rate and resistance to starvation, neither of which are maximized at the estimated position of the intake target.

Finally, while our consideration of performance axes to this point has focused on the components of evolutionary fitness, this approach can of course be used to link nutritional biology and applied animal and human sciences. Performance axes might, for example, be used to define agricultural management or human health targets and evaluate in this context the responses of experimental subjects to various nutritional regimes, in a manner analogous to the models of Parks (1982). It might thus be desirable to devise a diet which maximizes the milk yield of dairy cattle, or optimizes its composition, and the effects of the balance of nutrients on these variables might be quantified using a performance axis. Equally, welfare considerations may be taken into account. For example, if the composition of the diet which best achieves the manager's objectives does not correspond with the point of intake selected under *ad lib.* conditions, it may be desirable from a welfare perspective to shift the selected point of intake through selective breeding.

Perspectives

The past 50 years have witnessed impressive advances in the understanding of behavioural, physiological and ecological aspects of nutritional regulation in both vertebrate and invertebrate animals. These are, in no small part, due to the ability of researchers to dissect with increasingly fine resolution the individual components (e.g. molecular, physiological, neural and behavioural) of regulatory systems, and we predict that the future will see a welcome continuation of this trend. However, animals are extremely complex integrated systems, whose regulatory abilities are critically dependent on the details of the interactions among the sub-components. We have therefore taken the approach in our research into insect nutrition that increased understanding of nutritional subsystems should be accompanied by increased impetus to understand how the various components interact. In this review we have presented a conceptual approach which has proved useful in integrating the data and directing research for insects, and have attempted to extend these to a re-analysis of data from the relatively more complex vertebrate systems. It is hoped that researchers of vertebrate nutrition might find some inspiration in this approach, ultimately providing data which can further the understanding of the similarities and differences among vertebrate and invertebrate nutritional systems.

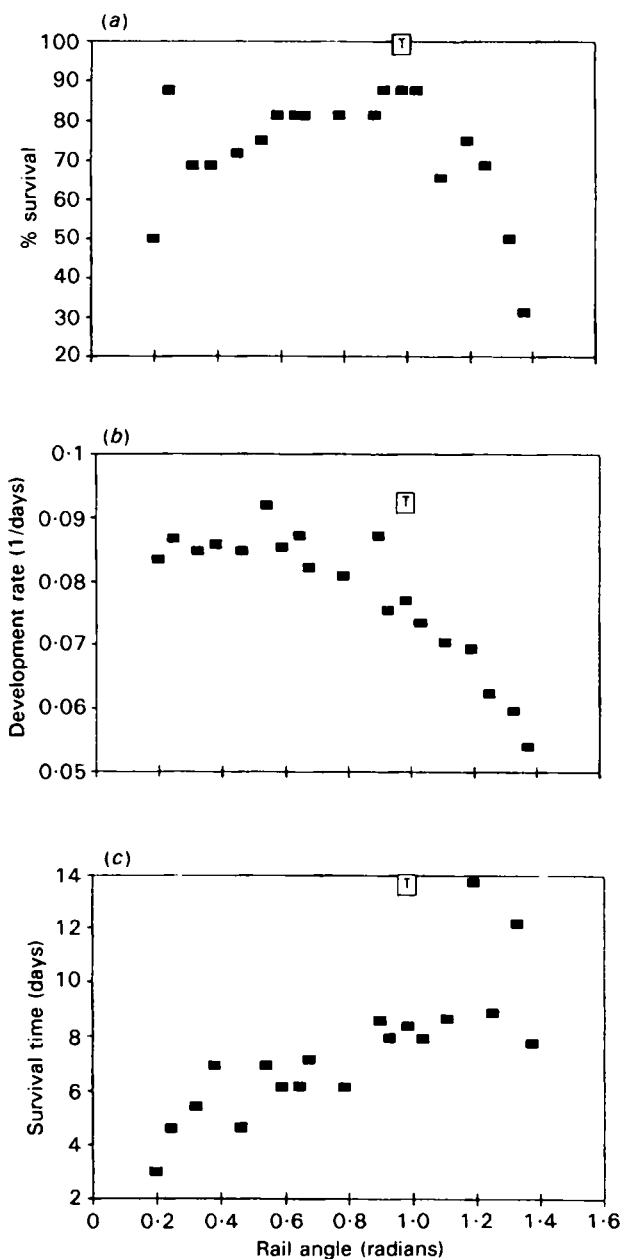


Fig. 19. Relationship between performance and the protein:carbohydrate ratio of synthetic foods (rail angle) fed to *Locusta migratoria* throughout the fifth larval stadium. (a) The proportion of locusts surviving through the fifth stadium. (b) The rate of development from the beginning of the fifth stadium to the subsequent moult (i.e. into adults). (c) The time to death by starvation for unfed adults which had been raised through the fifth stadium on the various experimental foods. Data from D. Raubenheimer & S.J. Simpson, in preparation.

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