

The Mechanisms of Nutritional Homeostasis

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9.1. Introduction

Insects have evolved means of using an extraordinary variety of food sources, many of which are, to say the least, nutritionally unpromising. In addition to possessing an impressive array of mechanisms to deal with the physical, chemical, and other challenges posed by such foods, it has become evident in recent years that insects can also cope with two additional problems: the heterogeneity of available diets and their own changing nutritional needs. Compensatory responses are exhibited which help to alleviate the deleterious effects of such nutritional discrepancies. Such responses can be both behavioral (through food selection and regulation of amounts eaten) and postdigestive. The properties of foods which play a role include not only nutrients, both singly and interactively (Simpson and Raubenheimer, 1993a,b; Simpson and Simpson, 1990; Slansky, 1993; Slansky and Wheeler, 1991; Waldbauer and Friedman, 1991; Wheeler and Slansky, 1991), but also water (Bernays, 1990; Raubenheimer and Gade, 1993, 1994) and the combination of nutrients and allelochemicals (Raubenheimer, 1992; Raubenheimer and Simpson, 1990; Slansky, 1992; Slansky and Wheeler, 1992).

The behavioral and underlying physiological mechanisms with which insects achieve nutritional homeostasis have been studied intensively in recent years. The subject was reviewed for phytophagous insects by Simpson and Simpson (1990), while Waldbauer and Friedman (1988, 1991) provided a detailed overview of dietary selection. Following the publication of these reviews there have been advances in our understanding of the proximate mechanisms controlling nutritional homeostasis and also in the way in which nutrition is viewed in functional terms (Raubenheimer, 1992; Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993a,b).

In this chapter we review nutritional homeostasis in the light of the more

recent work. In particular, we address the multidimensionality of nutritional needs and the interactions occurring between the physiological components which simultaneously regulate intake and utilization of a range of different nutrients.

9.2. The Geometry of Compensation

9.2.1. Targets

Much of this and the chapter by Barton Browne (Chapter 11) is based on the idea that meeting the nutritional requirements of an animal can be viewed as a problem of multidimensional geometry (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993b). At any instant there is a particular quantity and blend of nutrients which, if provided to the tissues, would maximize the animal's fitness. This is defined as the *nutritional target*. It is a "global" optimum (i.e., the best possible outcome, without further evolution) in the interaction between the genotype and the environment in which that genotype evolved (Raubenheimer and Simpson, 1994). The target lies in an n -dimensional "nutrient space," where n is the number of different nutrients needed.

The position of the nutritional target moves as the requirements of the tissues for nutrients change (see Chapter 11). Just as the needs of the tissues change with time, so too may the sources of nutrients available to the animal. The insect is therefore faced with the problem of matching the uncertainty of nutrient availability with changing nutritional requirements, both of which may move in n dimensions.

Approaching the nutritional target involves two stages: feeding and post-ingestive processing of food. The *intake target* is defined as the amount and blend of nutrients that must be ingested for postingestive processing to act at optimal efficiency, and thus reach the nutritional target.

There is also a third target: the *growth target*. This is the point in nutrient space which represents the optimal quantity and mix of nutrients required to build new tissue (somatic, reproduction, storage). The growth target will be separated from the nutritional target by the amount of nutrients needed to fuel metabolism.

9.2.2. Reaching the Intake Target: Nutritional Rails, Points of Best Compromise, and Behavioral Rules

A single food item, consisting of a fixed proportion of various nutrients, can be thought of as providing a "rail" in multidimensional nutrient space. The analogy of the rail illustrates the fact that an animal eating one food can only move in nutrient space along the trajectory representing the ratio of nutrients in that food. If the food contains two nutrients, A and B, in the ratio 1:2, then every mouthful ingested will provide twice as much B as A. The animal can "decide" how far

along the rail to go (i.e., how much to eat), but it can only leave the rail by differentially utilizing ingested nutrients (i.e., through postingestive compensation; see Section 9.4) or by choosing a different food.

Unless the rail passes through the intake target (i.e., the food is perfectly balanced), the intake target is unreachable for an animal restricted to that food. Even if, as is usually the case, there is a choice of different foods available, the intake target will still be unreachable if it lies outside the nutrient space bounded by the rails for the various food items (Fig. 9.1). When the intake target cannot be reached, there will be a *point of best compromise*, which is the amount and ratio of nutrients which, within the nutrient space available, provides maximal fitness when ingested. Each rail will bear its own point of best compromise (intra-rail optimum). The array of such points across a selection of rails within the nutrient space will provide a pattern that can be described by a particular behavioral rule (Fig. 9.2).

The point of best compromise is determined not only by the nutritional composition of the food, but also by such factors as the energetic and ecological costs of acquiring and processing food and the presence of allelochemicals (Hinks et al., 1993; Martin and Van't Hof, 1988; Raubenheimer, 1992; Slansky, 1992, 1993).

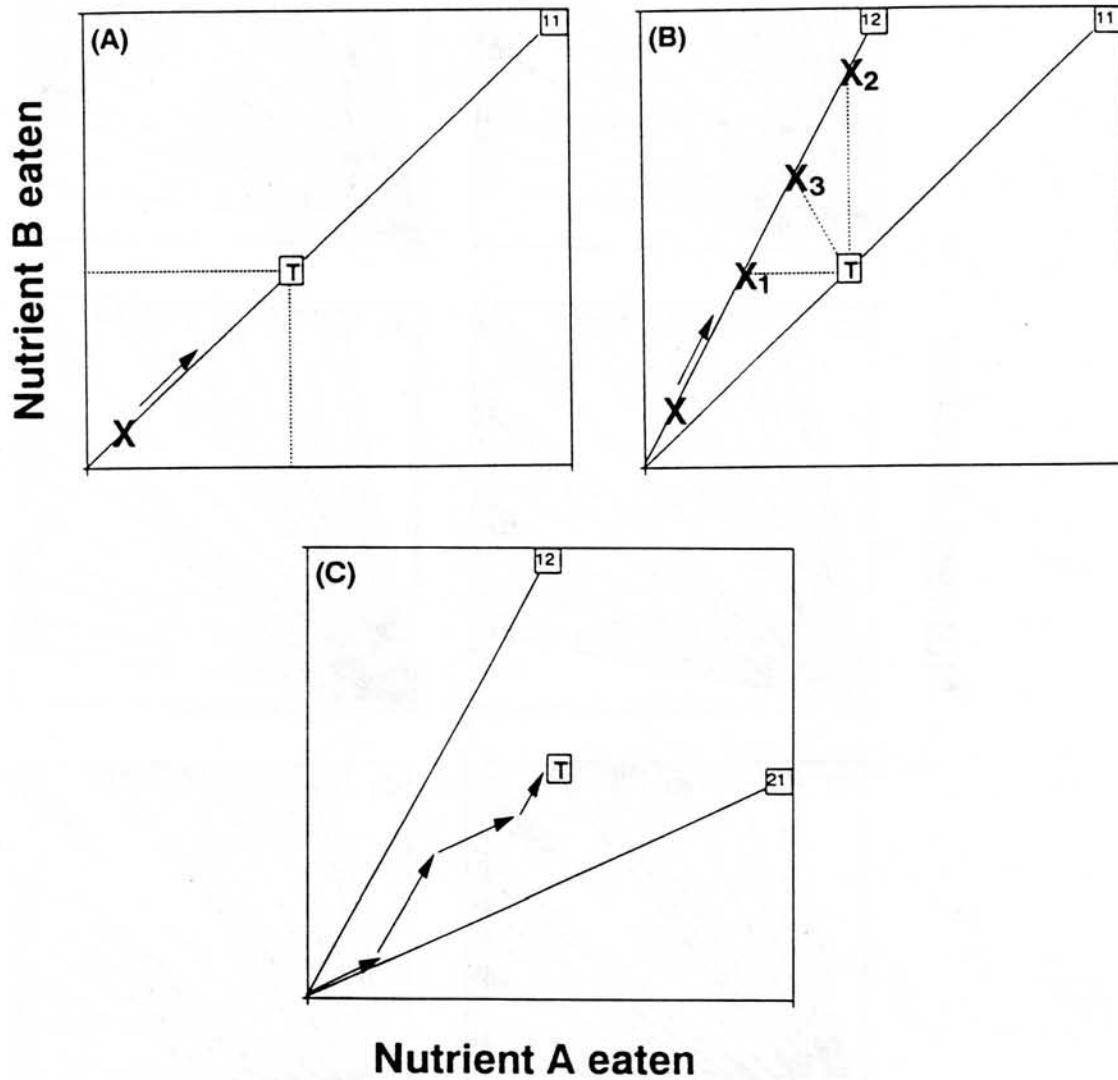
9.2.3. Reaching the Nutritional and Growth Targets: Leaving the Rails

Although an insect with a restricted choice of foods is only able to reach certain parts of the multidimensional nutrient space by altering feeding behavior, it is, in theory, able to move over a much wider region by differentially utilizing ingested nutrients. This could occur at the level of the gut, or once nutrients have entered the hemolymph. In this way the animal may be able to reach the growth target, despite not having been able to reach the intake target. Such postingestive mechanisms are discussed further in Section 9.4.

9.2.4. Experimental Data

The concepts described so far were developed and tested in an experiment on fifth-instar nymphs of the locust *Locusta migratoria* (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993b). This was similar to the hypothetical experiment shown in Fig. 9.2. Locusts were provided with one of 25 artificial foods, containing one of five levels each (7%, 14%, 21%, 28%, and 35%) of protein and digestible carbohydrate in an otherwise nutritionally similar mix. Consumption and growth were measured over the stadium.

The position of the growth target was estimated from plots in which growth over the stadium was separated into two components: that derived from ingested protein and that from ingested carbohydrate (Fig. 9.3). The position of the intake target was independently estimated in three ways, by: (a) using performance



Nutrient A eaten

Figure 9.1. Nutrient planes for intake of two nutrients, A and B. Amounts eaten of A and B are plotted against each other, with the ratio of A to B in each food being shown in the boxes at the end of each rail. The first and second digits of this number give the level in the food of A and B, respectively. The intake target (T) lies on the rail-bearing foods containing an A-to-B ratio of 1:1. (A) Graph showing how an animal on a food containing the optimal A-to-B ratio can reach the target from its current position (X). The dry weight of food which must be eaten to reach T will depend on how dilute the food is. (B) The animal is given a food containing an A-to-B ratio of 1:2, in which case T is unreachable. Instead the animal might (a) move along the rail until the target coordinate for B is reached (X_1), and thus suffer a shortfall in A, (b) eat until the coordinate for A is reached (X_2), and thus overeat B, or (c) move to an intermediate point (X_3). (C) Graph showing 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 thus reach the target. (After Simpson and Raubenheimer, 1993b.)

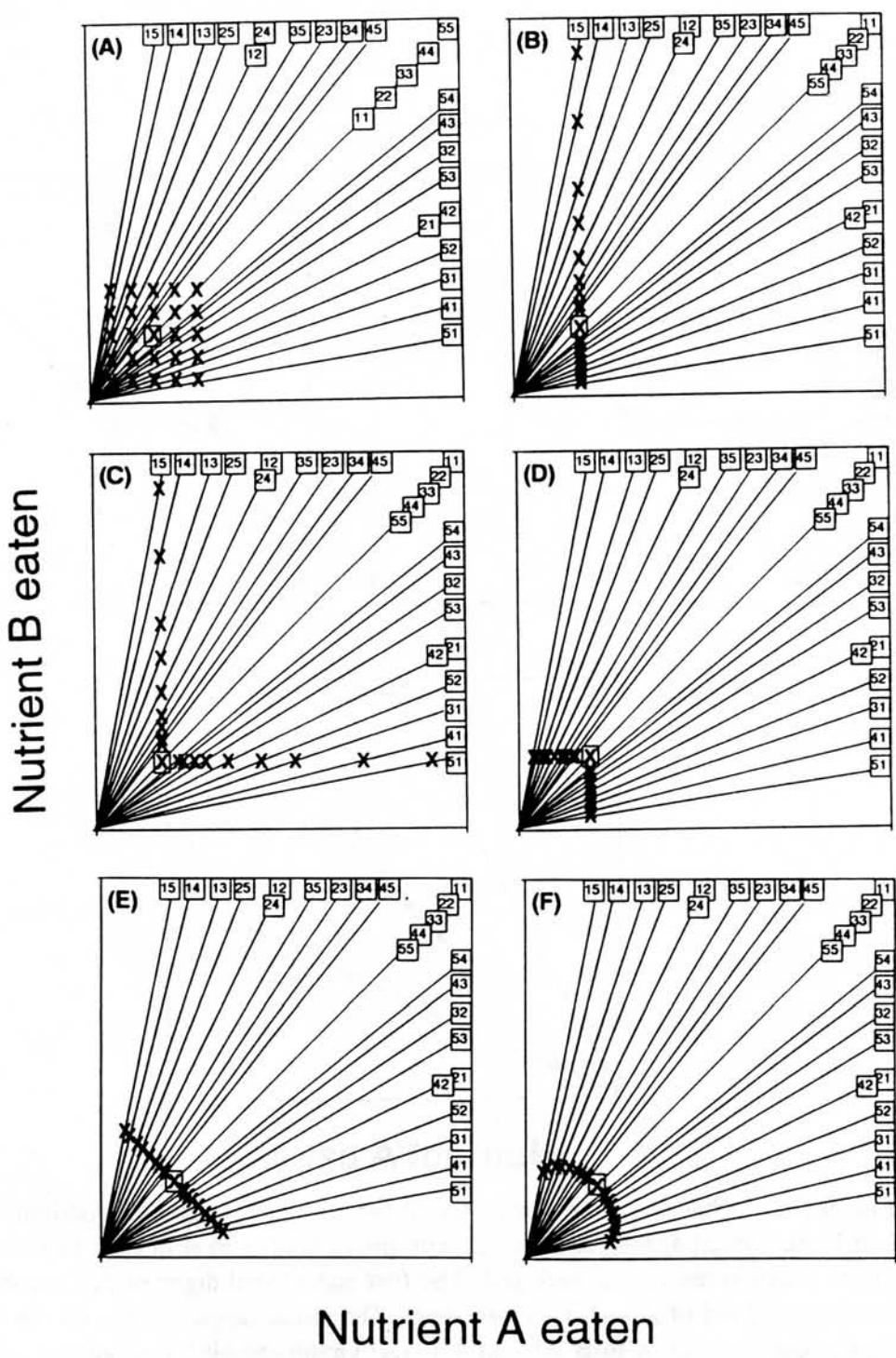


Figure 9.2. Nutrient planes from a hypothetical study in which animals were fed one of 25 foods, containing one of five levels each of two nutrients, A and B. Each of the panels indicates the array arising from a particular functional feeding rule. The intake target is shown as a square in each panel.

- (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 is reached for A, irrespective of how much B is consumed.
- (C) **Rule 3:** Eat until at least the intake target level of both A and B is reached.
- (D) **Rule 4:** Eat until the intake target level of either A or B is reached.
- (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 geometrically closest distance to the intake target is reached on the rail (“closest distance optimization”). (After Simpson and Raubenheimer, 1993b.)

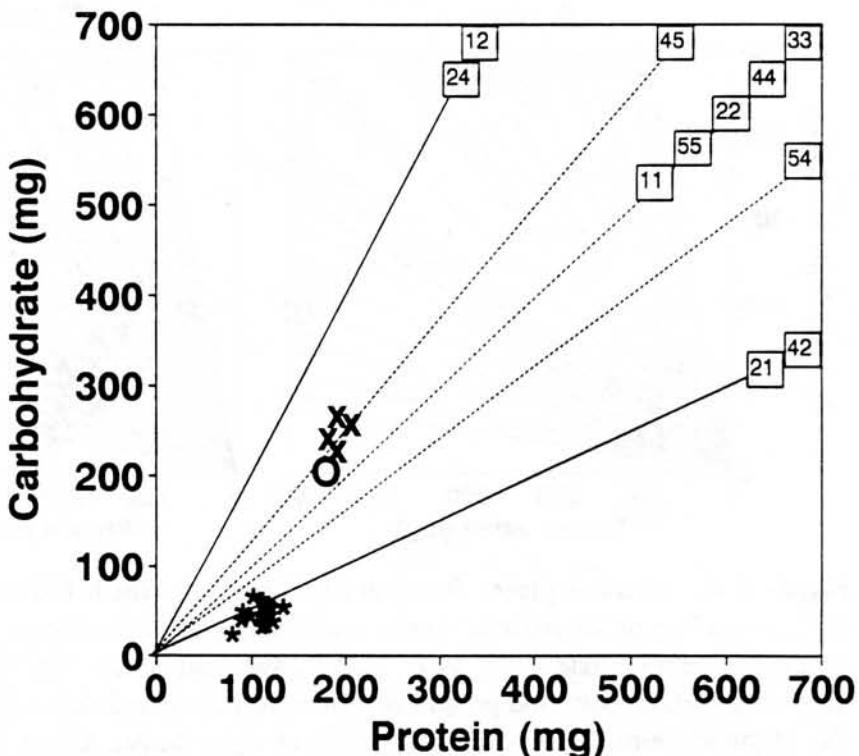


Figure 9.3. Nutrient plane for protein and carbohydrate in fifth-instar nymphs of *Locusta migratoria*. The asterisks indicate growth points (bicoordinate points of growth derived from ingested protein and carbohydrate) reached by locusts fed a wide range of artificial foods varying five-fold (7–35%) in protein and carbohydrate content in no-choice experiments. Note how they are tightly clustered, indicating the position of the growth target. Some of the 25 foods used are shown in the boxes at the end of the rails. The percentage of protein and carbohydrate in the foods can be derived by multiplying the first and second digits by 7. The crosses indicate the amounts of nutrients consumed by locusts provided with a choice of foods (protein, carbohydrate), 1,2 or 2,4, with 2,1 or 4,2 in a separate experiment (see Chambers et al., 1995). The closely grouped points indicate the likely position of the intake target. The circle shows another estimate of the intake target derived from the growth target and published values for metabolic costs and digestive asymmetries. (After Simpson and Raubenheimer, 1993b.)

criteria (minimum mortality and development time), (b) calculating back from the growth target, using published values for respiration and digestive efficiencies, and (c) allowing insects to select and defend a point on the nutritional plane. The latter was achieved by providing nymphs with one of a series of different two-diet choices (Chambers et al., 1995; Simpson et al., 1988). There was very close agreement between the different estimates for the position of the intake target for protein and carbohydrate, with the target lying close to the rail representing a protein-to-digestible-carbohydrate ratio of 45:55 (Fig. 9.3).

Plots of intake of carbohydrate and protein over the main feeding period in the stadium showed markedly arc-like arrays (Fig. 9.4), very similar to that described by the behavioral rule closest distance optimization (Fig. 9.2F). To

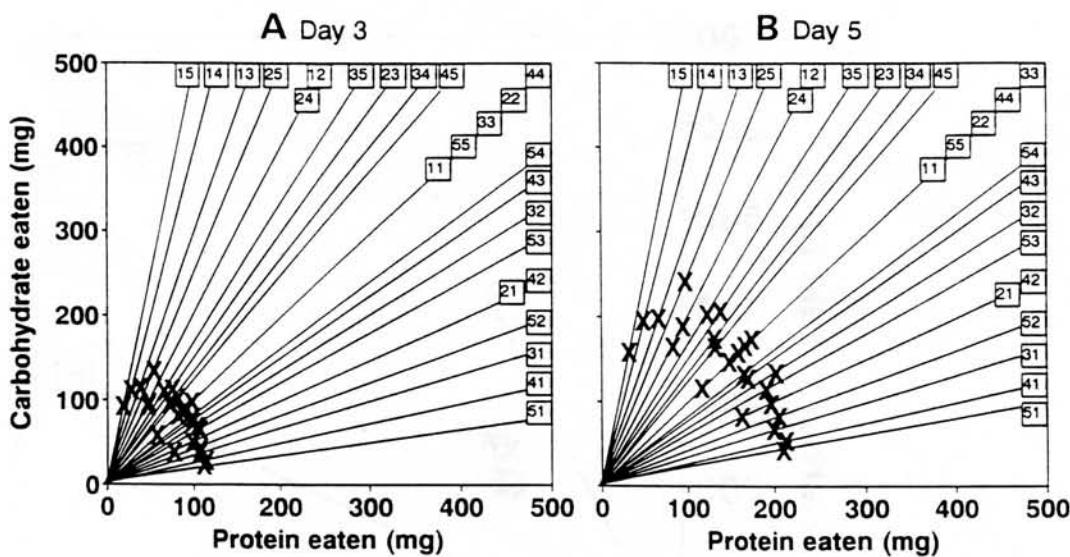


Figure 9.4. Nutrient planes from an experiment in which fifth-instar locust nymphs were given one of 25 artificial foods, containing one of five levels each of protein and digestible carbohydrate (7%, 14%, 21%, 28%, and 35%). The cumulative intake of digestible carbohydrate and protein is shown over the first 3 days (**A**) and the first 5 days (**B**) of the stadium. Each cross is the mean of eight locusts. Given that the intake target lies close to the mid-rail (Fig. 9.3), this array is closely similar to that described by functional rule 6, the closest distance optimization (see Fig. 9.2F). (After Raubenheimer and Simpson, 1993.)

provide such patterns the insects had eaten considerably different dry weights of the various foods, this being especially evident where foods lay on the same rail but were more or less diluted by addition of cellulose. Reaching the same growth point from the disparate points of protein and carbohydrate intake meant that locusts on different foods had differentially utilized protein and carbohydrate post-ingestively (see Section 9.4).

An analysis of published data from larvae of a number of other insect species (Simpson and Raubenheimer, 1993b) showed that the position of the intake target for protein and carbohydrate differs widely according to life-history characteristics such as possession of mycetocyte symbionts and whether or not the insects fed as an adult. All data were nevertheless consistent with the same behavioral rule: closest distance optimization (Fig. 9.5). A study of first-instar pea aphids, *Acyrthosiphon pisum*, was also compatible with this rule (Fig. 9.6), although, because the intake target lay very close to the carbohydrate axis (a dry-weight amino-acids-to-sugar ratio of 6:94 provided maximum performance) and only a narrow range of possible combinations of sugar and amino acids was physiologically reasonable to test, the rule shown in Fig. 9.2B described the recorded intake pattern equally well (Abisgold et al., 1994). The latter rule also represented the relationship between dietary salt and nonmineral nutrients in locust nymphs, where the intake target salt-to-nonmineral-nutrients ratio was 6:94 (Trumper and Simpson, 1993).

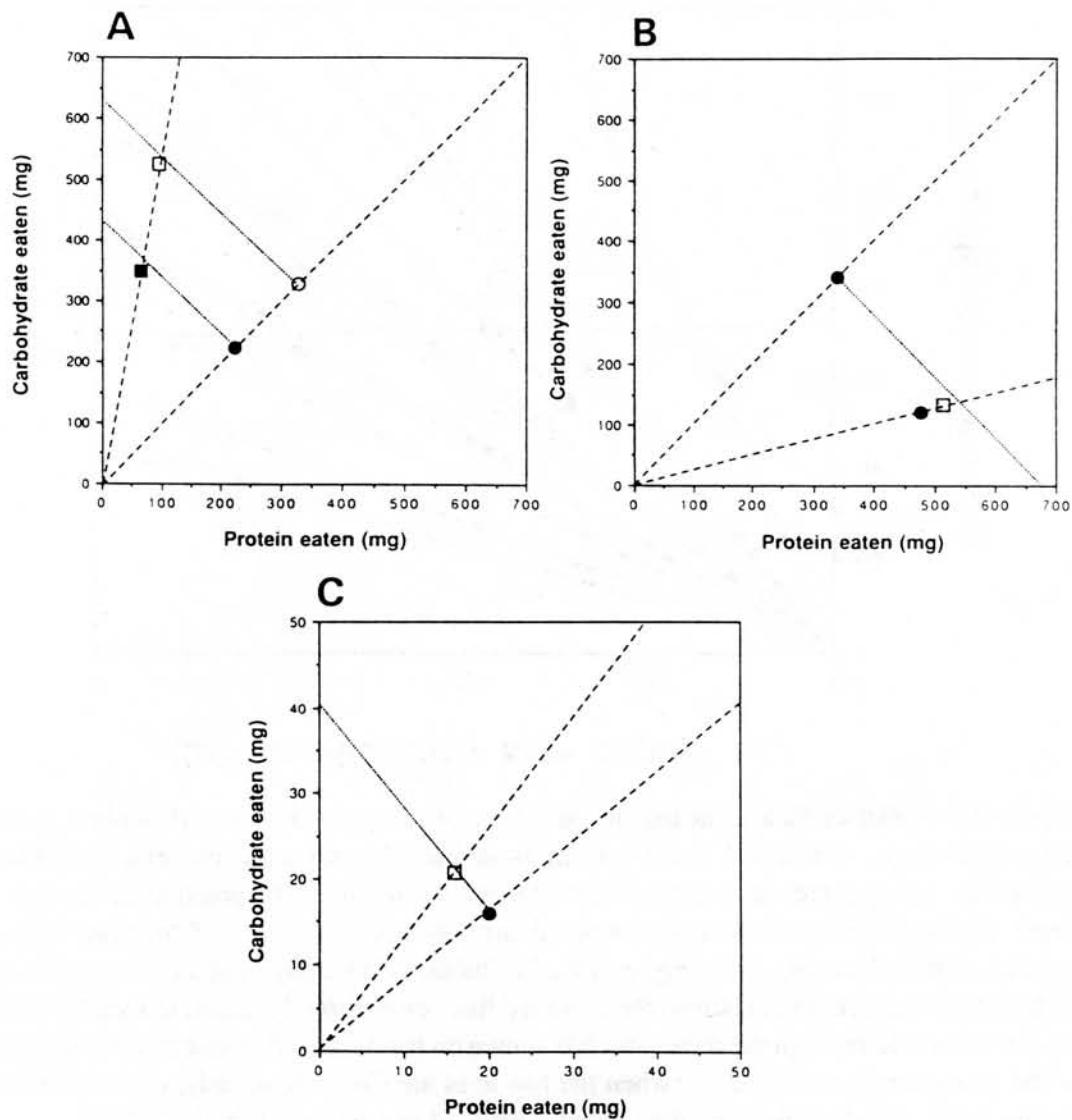


Figure 9.5. A reanalysis of published data for protein and carbohydrate intake. (A) Nymphs of the brown-banded cockroach, *Supella longipalpa*. (Data from Cohen et al., 1987.) (B) Caterpillars of *Helicoverpa zea*. (Data from Waldbauer et al., 1984). (C) Larval *Tribolium confusum*. (Data from Waldbauer and Battacharya, 1973). In each case the squares indicate the average point reached by a cohort of insects given a choice of two foods, while the circles show the points reached when the insects were fed only one food [in the case of *H. zea* there are two such points, one for a food containing (protein, carbohydrate) 1,1 and another 8,2 (the self-selected ratio)]. In the graph for *S. longipalpa*, data are shown for insects tested over the entire larval period (open symbols) and the last half of larval development (closed symbols). Note how the position of the self-selected point varies between the species, being protein-biased for the caterpillar (which feeds for much of its adult as well as its larval protein needs), carbohydrate-biased for the cockroach (which possesses mycetocyte symbionts implicated in upgrading dietary nitrogen), and close to the mid-rail for the beetle (which feeds as an adult and lacks mycetocyte symbionts). Additionally, note how in each case a line drawn at right angles to the rails at the points reached by insects given no choice of food passes close to the selected point. This is consistent with closest distance optimization, if it is assumed that the selected point is the intake target. (After Simpson and Raubenheimer, 1993b.)

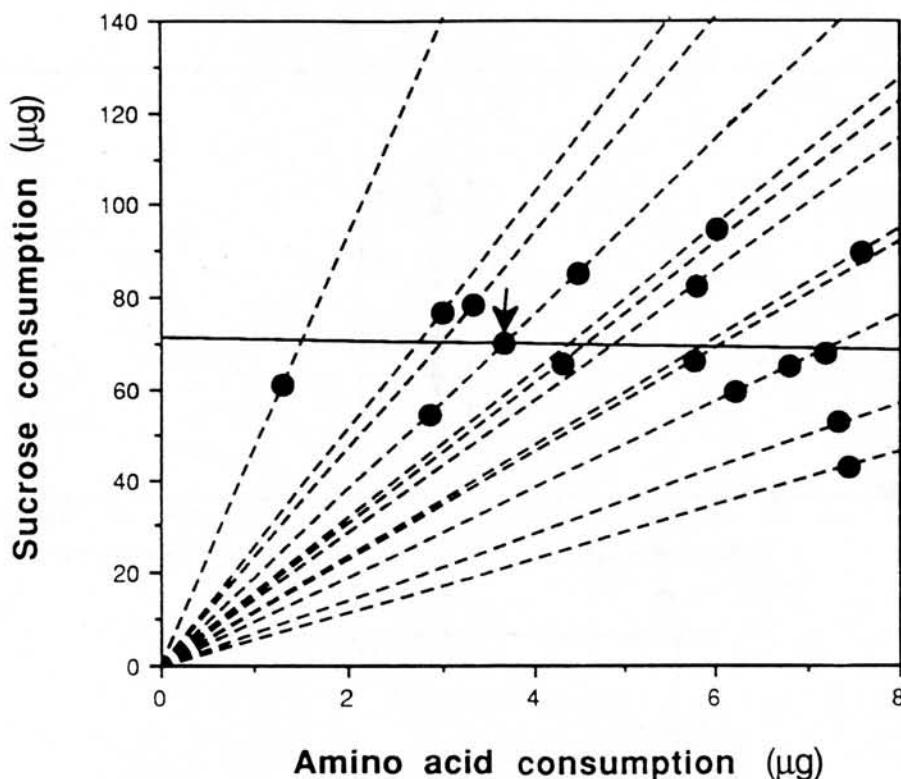


Figure 9.6. Plot of data from first-instar larvae of the pea aphid *Acyrthosiphon pisum* fed a wide range of artificial foods varying in amino acid and sugar content. Note that, unlike Fig. 9.5, the axes are scaled differently from each other. The position of the intake target (derived from performance criteria) is arrowed and is highly carbohydrate-biased (aphids, like cockroaches, have mycetocyte symbionts). The array of intakes is consistent with closest distance optimization (the expected line being virtually indistinguishable from the line of best fit through the data, which is shown on the figure). Because of the closeness of the intake target to the y-axis (when the two axes are similarly scaled), the rule shown in Fig. 9.2B also describes the data equally well. (After Abisgold et al., 1994.)

9.3. The Physiological Control of Nutrient Intake

Approaching the intake target or point of best compromise in nutrient space involves regulating the type of food selected and also the amounts of each food eaten. These are not alternatives but are different stages in the regulation of nutrient intake, and as such they share certain controlling mechanisms. These mechanisms direct the insect through nutrient space by integrating information about the current nutritional state of the animal and the nutritional quality of the food (Simpson and Raubenheimer, 1993a).

While insects can gain information about the nutritional quality of food by using chemoreceptors innately tuned to various nutrients (Bernays and Simpson, 1982; Simpson and Simpson, 1990; Trumper and Simpson, 1993; also see Chapter 4), and perhaps even chemoreceptors in the gut (Champagne and Bernays, 1991; Timmins and Reynolds, 1992; see also Deutsch, 1990), these inputs alone cannot

provide a mechanism for nutritional homeostasis unless they are integrated with information about the insect's current nutritional state.

9.3.1. Assessing Nutritional State

Data from a range of insect species have indicated a central role for the hemolymph in nutritional homeostasis (reviewed by Simpson and Raubenheimer, 1993a). Hemolymph composition varies with time since a meal, with quantity and quality of previous meals, and with the metabolic and growth demands of the tissues. As a result, the hemolymph has the potential to provide constantly updated information about the insect's nutritional state. That such information is used in nutritional homeostasis is evidenced by the fact that feeding behavior is affected by blood titers of specific nutrients, notably amino acids and sugars (Abisgold and Simpson, 1987, 1988; Friedman et al., 1991; Simpson and Simpson, 1992), and also by hemolymph osmolality (Abisgold and Simpson, 1987; Bernays and Chapman, 1974; Gelperin, 1966). The latter indicates the combined solute concentration of the hemolymph. Although adequate for regulating feeding in an insect such as a male blowfly, which is feeding primarily to meet energetic demands (i.e., has a one-dimensional nutrient space), general properties such as osmolality are not adequate indicators of the multiple needs of a growing insect.

9.3.2. Linking Nutritional State to Behavior

Feeding involves a sequence of behavioral components: finding food, accepting or rejecting a potential food item, and then ingestion. Hemolymph composition is known to influence each of these in several groups of insect, including blowflies, grasshoppers, and caterpillars (reviewed by Simpson and Raubenheimer, 1993a). How, then, is hemolymph composition linked to behavior?

9.3.3. Finding Food

Nutrient deficiency results in increased incidence of locomotion, which, in turn, enhances the probability of finding appropriate food. This is the case for insects which are deprived entirely of food (Barton Browne, 1975; Simpson and Simpson, 1990), fed nutritionally diluted diets (Naeem et al., 1992; Simpson et al., 1990), or given foods which lack adequate quantities of single nutrients within an otherwise complete mix (Cohen et al., 1987; Naeem et al., 1992; Trumper and Simpson, 1994). Similarly, insects which are provided with a nutritionally dilute food locomote sooner, on average, after a meal than when fed more concentrated foods (Simpson and Abisgold, 1985; Simpson et al., 1990).

Osmolality and levels of specific nutrients in the hemolymph play a role in such responses, along with declining inhibition from stretch receptors on the gut or body wall and falling titers of neurohormones released as a result of gut or body distention (Abisgold and Simpson, 1987; Bernays and Simpson, 1982;

Friedman et al., 1991). How these control locomotion is as yet unclear. Cohen et al. (1988) suggested that perturbations in levels of neurotransmitters in the central nervous system (CNS) may result from feeding on nutritionally imbalanced diets, perhaps as a result of low levels of precursors coming from the hemolymph, and lead to enhanced locomotion.

Not only is the likelihood of locomoting affected by nutritional state, so too is the direction of locomotion, and instances are known where orientation occurs toward stimuli emanating from foods containing specific nutrients required at that time (e.g., Robacker, 1992; Simpson and White, 1990). In nymphs of *Locusta migratoria*, learning is involved here (Simpson and White, 1990; see Section 9.3.6).

9.3.4. Accepting or Rejecting Food

In most insects the initiation of feeding is dependent upon appropriate input to the CNS from chemoreceptors responding to the food (Bernays and Simpson, 1982). It is now well known for locusts and caterpillars (but disputed for blowflies) that the responsiveness of such receptors is not fixed but varies with the nutritional state of the insect (Abisgold and Simpson, 1988; Blaney et al., 1986; Simmonds et al., 1992; Simpson et al., 1990b; Simpson and Simpson, 1992; Simpson et al., 1991). This means that insects can anticipate the nutritional suitability of a newly contacted food without first having to ingest it and then rely on postigestive feedbacks.

How such responses are mediated is best understood in nymphs of *Locusta migratoria* (Abisgold and Simpson, 1987, 1988; Simpson et al., 1990a; Simpson and Simpson, 1992; Simpson et al., 1990, 1991). In this animal the responsiveness of mouthpart taste receptors to stimulation with amino acids and sugars changes with nutritional state, with chemosensitivity to the two nutrient groups being regulated independently. Similar, independent modulation of gustatory responsiveness to sugars and amino acids has also been described in *Spodoptera littoralis* caterpillars (Simmonds et al., 1992).

In locusts, peripheral responsiveness to amino acids is modulated by levels of eight key amino acids in the hemolymph (Abisgold and Simpson, 1988; Simpson et al., 1990a). Central neural or hormonal feedbacks are not required. Instead, blood amino acids modulate maxillary palp taste receptors at the periphery (Simpson and Simpson, 1992). It would seem that the taste receptors somehow measure the difference between levels of amino acids in the hemolymph and in (or on) the food.

Taste receptors do not exist for all required nutrients, in which cases the type of mechanism which contributes to regulation of amino acid and carbohydrate intake cannot operate. However, levels of amino acids and sugars in the hemolymph could still provide a means of ensuring that adequate quantities of other nutrients are eaten. Shortage or absence in the food of any required nutrient will

sooner or later cause a metabolic bottleneck within the insect and lead to elevated concentrations in the hemolymph of other nutrients which are present in the food at adequate concentrations.

For instance, shortage of a vitamin may eventually result in a buildup in amino acids and sugars which will "jam" feeding control systems, partly by causing desensitization of taste receptors (Friedman et al., 1991; Simpson and Raubenheimer, 1993a; Simpson and Simpson, 1990; Fig. 9.7). As the effect of the bottleneck becomes more pronounced, the likelihood that the insect will reject the food will increase. Rejection and subsequent locomotion will enhance the probability that the animal will move away and contact other, perhaps more suitable foods which will be accepted once hemolymph nutrient levels have declined following excretion, respiration, and so on.

In addition to direct modulation of gustatory receptors, other mechanisms are likely to link nutritional state with acceptance behavior by specifically varying central thresholds to chemosensory input. For instance, Okajima et al. (1989) discovered an internal receptor responding to hemolymph concentrations of trehalose in larval *Mamestra brassicae*, and central neurones responding to nutrients are well known in vertebrates (e.g., Gietzen, 1993; Karadi et al., 1992; Oomura, 1988).

9.3.5. Ingestion: Regulation of Meal Size

The size of meal eaten of a given food is affected by an insect's nutritional state. This has been demonstrated in larval *L. migratoria* and *S. littoralis* following pretreatment on artificial foods lacking either protein or carbohydrate. Here meals, if they began at all (Section 9.3.4), were considerably smaller on foods lacking the deficient nutrient than on those containing it (Simmonds et al., 1992; Simpson et al., 1988, 1990, 1991). Experiments with these two species, and with larvae of two other caterpillars, *Helicoverpa zea* (Friedman et al., 1991) and *Manduca sexta* (Timmings and Reynolds, 1992; Timmings et al., 1988), indicate a central role for hemolymph nutrients in the regulation of meal size, although the results from *M. sexta* could also be explained by chemoreceptors in the gut, if these exist. In male sheep blowflies, *Lucilia cuprina*, fed *ad libitum* on one of two concentrations of sugar solution, larger meals were taken of the dilute than of the concentrated solution (Simpson et al., 1990). This was explicable in terms of a nonspecific effect of the concentration of sugar on hemolymph osmolality (Gelperin, 1966; Simpson et al., 1990).

Nutritional state, as represented by osmolality or nutrient levels in the hemolymph, could potentially influence the amount of food ingested during a meal in one or both of two ways. First, high levels of nutrients remaining from previous meals might provide inhibition which results in the meal being terminated sooner than if blood nutrient levels were low when the meal began. Second, nutrient levels might rise during the current meal and augment other inhibitory inputs

which terminate feeding, such as volumetric feedbacks. Both are likely to occur, and in the case of *L. migratoria* and *S. littoralis* they are known to involve modulation of gustatory responsiveness (Simmonds et al., 1992; Simpson et al., 1991).

9.3.6. Learning

The emphasis in the discussion so far has been on the direct feedback mechanisms which regulate nutrient intake. However, it is becoming increasingly apparent that, as in vertebrates (Booth, 1991; Rozin, 1976), learning also plays a major role and that a combination of learned responses and direct feedbacks allows considerable behavioral flexibility (Figs. 9.7 and 9.8; see Chapter 10). The balance of the two in the control of behavior is likely to vary according to the type of insect and the nature of its nutritional environment (Bernays and Bright, 1993).

Learned responses described to date involving nutrients have been of several types: positive associations (Raubenheimer and Blackshaw, 1994; Simpson and White, 1990), food aversions (Bernays, 1993; Champagne and Bernays, 1991) and nonassociative responses, such as neophilia (Bernays and Raubenheimer, 1991; Geissler and Rollo, 1988; Trumper and Simpson, 1994). Learning has been implicated in orientation toward nutritionally appropriate food cues, food acceptance, and the control of meal size. The unconditioned stimuli in learned associations are as yet unclear, but are likely at some level to involve hemolymph composition (Simpson and Raubenheimer, 1993a).

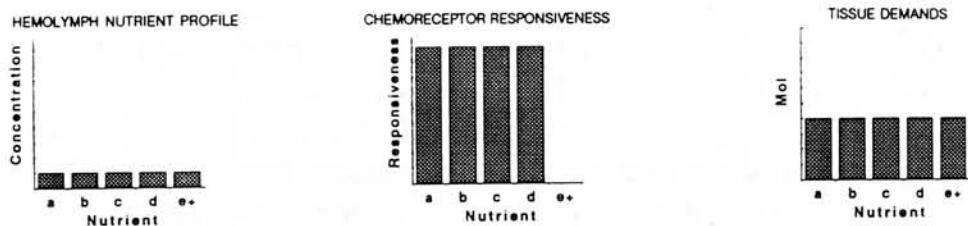
9.3.7. A Model for Nutritional Compensation

Figure 9.7 shows a graphical summary of a model for the control of compensatory feeding, based on data from locusts and caterpillars. The y-axes of all graphs are to an arbitrary scale of 1–9.

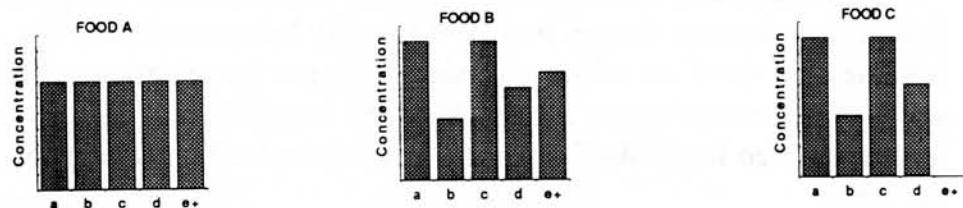
The top line of graphs shows the state of the insect immediately before it contacts a potential food item. The left graph shows the concentration of nutrients in the hemolymph. For a–d there is gustatory responsiveness. Nutrients e+ are those for which there is no gustatory responsiveness. The middle graph shows the level of gustatory responsiveness to nutrients in the food. The level for each nutrient is a reciprocal function of the blood titer of that nutrient (except for e+, which are not detected). The right-hand graph indicates the requirements of the tissues for the nutrients at that time. Tissue demands are a function of developmental programs and environmental conditions and vary with time. In a full model incorporating a time axis, tissue demand would be represented as mol/time.

The second line shows the nutrient content of three foods. Food A is balanced with respect to the present demands of the tissues. Food B is imbalanced but

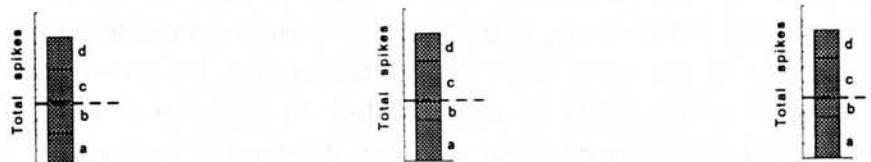
STATE OF INSECT IMMEDIATELY BEFORE CONTACTING FOOD



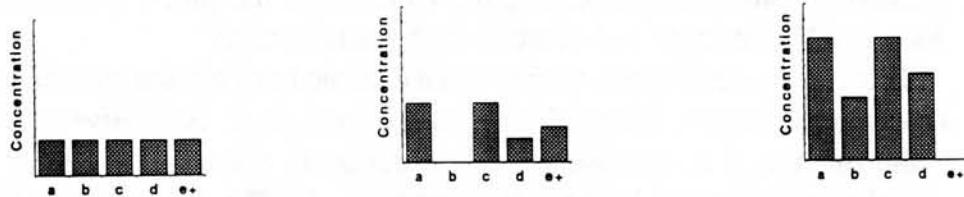
NUTRIENT PROFILE OF THE FOOD



PHAGOSTIMULATORY INPUT TO THE CNS ON SAMPLING FOOD



HAEMOLYMPH NUTRIENT PROFILE AT TIME t AFTER FEEDING



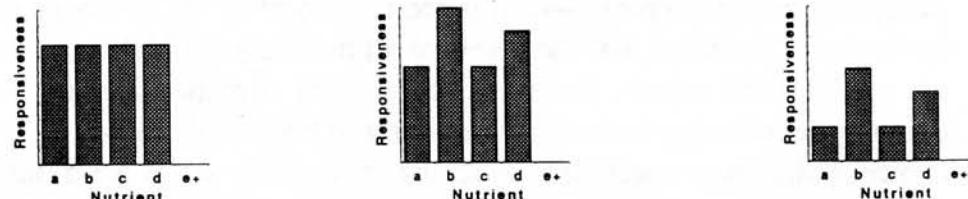
LEARNED ASSOCIATIONS WITH OTHER FOOD-RELATED STIMULI

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CHEMORECEPTOR RESPONSIVENESS AT TIME t AFTER FEEDING



PHAGOSTIMULATORY INPUT TO THE CNS IF SAMPLED FOOD AT TIME t

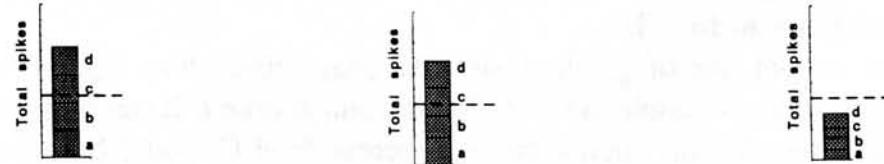


Figure 9.7. A graphical summary of a model for regulation of nutrient intake in insects. See text for explanation. (After Simpson and Raubenheimer, 1993a.)

not seriously deficient in any nutrient group. Food C has the same profile of nutrients a-d as does Food B, but lacks e+ entirely.

The third line shows the phagostimulatory input to the CNS when the insect samples one of the three foods. The number of spikes elicited by each of the nutrients a-d is a function of chemoreceptor responsiveness \times concentration in the food. The assumption is that spikes for a-d are of equal weighting and that they are simply summed in the CNS. Although other non-nutrient phagostimulants or deterrents are not shown, they could readily be incorporated. The dashed line indicates the threshold of input needed to trigger the initiation of feeding. Meal size is proportional to the excess above the threshold (see Chapter 5). Because in this instance foods A-C elicit the same spike total, meal size on all three is the same.

The fourth line shows the nutrient profile of the blood at time t after having taken a meal of foods A-C. The concentration of each nutrient is a function of amount ingested in the meal, minus tissue demands, minus amount egested and excreted. Both of the latter have been considered to be zero in the figure, but nonzero values could easily be incorporated. In the case of food C, the tissue demands could not be met for any nutrient, despite the presence of sufficient of a-d in the blood. This is because the absence of e+ in the food created a metabolic bottleneck preventing the nutrients in the blood from being utilized. As a result, nutrients a-d accrued in the hemolymph.

The fifth line indicates whether or not a learned association was made with other food-related stimuli, following feeding on foods A-C. A positive association was made on A and B because the tissue demands were met, whereas a negative association was made on the deficient food, C. The unconditioned stimulus is some function of blood nutrients.

The sixth line indicates chemoreceptor responsiveness at time t after feeding on foods A-C, calculated as described above. It can be seen here how feeding on the imbalanced, but nutritionally complete, food B leads to an asymmetrical pattern of responsiveness, which is the reciprocal of the levels of the nutrients in the food. Provided that the levels of all nutrients are high enough to support the needs of the tissues, this reciprocal pattern of responsiveness will result in the insect continuing to feed over a period of time on the food if given no other choice, rather than rejecting it as occurs for food C, which is seriously deficient in certain nutrients (see next line). It also means that if the insect encounters a food with higher levels of the more limiting nutrients, it will choose that food in preference to food B.

The bottom line of graphs shows the phagostimulatory input to the CNS if the insect was to sample the same foods again at time t . It can be seen that foods A and B would still elicit feeding, whereas food C would be rejected before ingestion.

This is a highly simplified set of relationships. Nevertheless, it shows how complex nutritional decisions can be based on simple mechanisms. The same

components can be used to explain behavior under both choice and no-choice situations. It can explain, for instance, why more of a nutritionally dilute food is ingested over time than of a nutrient-rich food, yet why the insect will prefer the nutritionally rich food in a choice test against the dilute one.

Figure 9.8 summarizes the known and hypothetical mechanisms involved in nutritional homeostasis.

9.4. Movement in Nutrient Space Through Postingestive Processes

Differential utilization of ingested nutrients enables an insect to move across nutrient space from the point of intake toward the nutritional and growth targets. That this occurs has been demonstrated experimentally in locust nymphs (Raubenheimer, 1992; Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993b; Fig 9.3) and larval aphids (Abisgold et al., 1994) and can be inferred from a number of other studies [see, e.g., Karowe and Martin (1989) and references in Simpson and Simpson (1990) and Slansky (1993)].

9.4.1. Nutrient Budgets

An understanding of postingestive processes involves the derivation of nutrient budgets. Budgets which investigate individual nutrient groups (e.g., proteins or carbohydrates, or individual compounds) have been termed discriminatory budgets (Raubenheimer and Simpson, 1994, 1995). Unitary budgets, by contrast, treat the sum of all nutrients as a single variable. Commonly, such budgets include all ingesta, even non-nutritional material such as cellulose. Unitary budgets are of limited interest because they fail to discriminate among physiological processes (Simpson and Simpson, 1990).

Raubenheimer and Simpson (1994) present a structure and terminology for nutrient budgets which allows the basic terms to be grouped according to either methodological or functional criteria. Using a functional classification, the discriminatory budget for a given nutrient, n , over time t can be stated in its most condensed form as:

$$I_n = U_n + W_n \quad [1]$$

where I is the amount of n ingested, U is the amount utilized by the animal for maintenance, behavior, growth, and so on, and W is wastage, due to ingested nutrients either being inaccessible to digestion and absorption or being in excess of metabolic requirements.

The terms W and U can be expanded to varying degrees, depending on the interests of a specific study [see Raubenheimer and Simpson (1994) for a full discussion]. The component terms of W and U are labeled using a system of

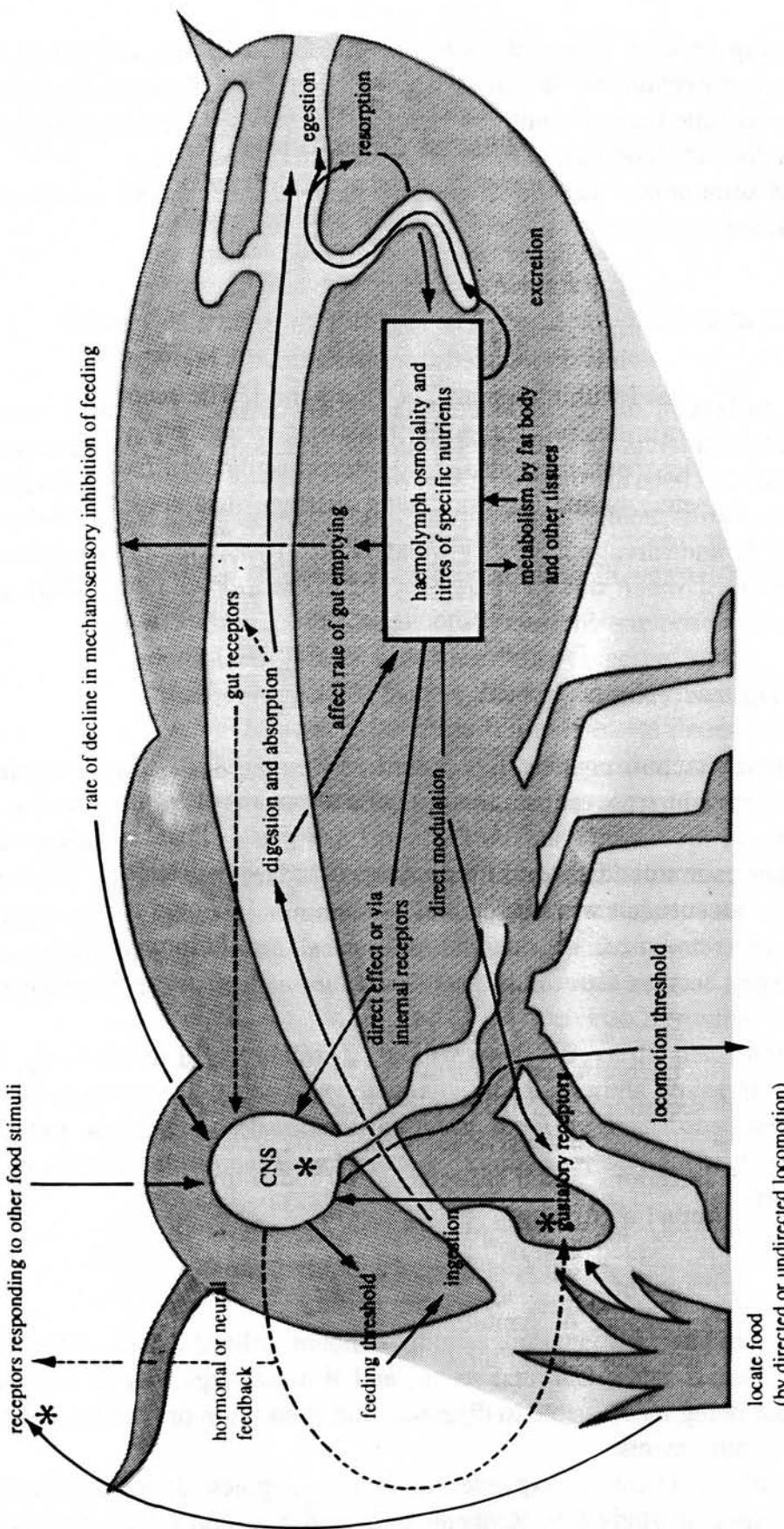


Figure 9.8. A summary indicating known and hypothesized (dashed lines) control mechanisms underlying nutritional homeostasis in a composite insect (based on acridids, caterpillars, and blowflies). At the center lies the composition of the hemolymph, which is influenced by nutrients coming from the gut, excretion, resorption from the hindgut, and metabolism by the fat body and other tissues. Hemolymph osmolarity affects the rate of gut emptying, and hence the rate at which volumetric inhibition of feeding and its consequent effects (e.g., stretch-induced release of hormones affecting locomotion and gustatory sensitivity) decline after the last meal. Additional information regarding food in the gut may come from chemoreceptors in the gut lumen. Hemolymph osmolarity, and perhaps also concentrations of specific nutrients, influences central neural thresholds for locomotion and feeding, either by acting via internal receptors or by affecting the balance of neurotransmitters in the CNS. Specific nutrient feedbacks for amino acids and sugars directly and independently modulate gustatory responsiveness, thereby altering the probability of accepting a food or terminating a meal. Further nutrient-specific modulation of sensory receptors may come from hormonal or centrifugal neural feedbacks. Asterisks indicate possible loci for learning associations between food-related stimuli and nutritional state; these could be central or peripheral.

symbols which indicates their logical status in the budget. For example, the terms from equation [1] might be expanded as:

$$W_n = (Deu + Ded + Dex + "Dm)_n + ("Rs + "Rp)_n \quad [2]$$

and

$$U_n = ("Dm + Dc)_n + (Rc + Rg + Rr + "Rs + "Rp + Ri)_n \quad [3]$$

where D denotes a component which is dissociated from the animal's body, and R is a component which is retained within the relevant time period. The second letter in each term distinguishes various subcategories of dissociated and retained nutrient (e.g., e is egested nutrient, m is metabolized, s is stored, and p is utilized by parasites and symbionts, etc.), while the third letter distinguishes subsets nested within these terms. Superscripted w and u denote, respectively, the wastage and utilized components of terms which occur both in W and U .

Thus, from equation [2]:

$Deu = n$ egested without having been digested;

$Ded = n$ egested having been digested but not absorbed across the gut;

$Dex = n$ catabolized and excreted;

$"Dm = n$ respired as a means of removal (i.e., a wastage component of respiration);

$"Rs =$ stored n which does not contribute to fitness (e.g., obesity);

$"Rp = n$ lost to parasites associated with the animal.

And, from equation [3]:

$"Dm = n$ respired to meet the energetic requirements for maintenance;

$Dc = n$ incorporated into secretions which are dissociated from the animal (e.g., peritrophic membranes, defensive secretions, silk, etc.);

$Rc = n$ incorporated into secretions which are retained by the animal;

$Rg = n$ incorporated into somatic growth;

$Rr = n$ incorporated into reproductive growth;

$"Rs = n$ stored for later utilization;

$"Rp = n$ allocated to the maintenance of symbionts;

$Ri = n$ interconverted to meet shortfalls in the budgets of other nutrients.

The sum of the term R_i across discriminatory budgets for all nutrients constitutes a common metabolic pool from which individual budgets draw (Rabenheimer and Simpson, 1994, 1995).

Relating these terms to nutritional targets (Section 9.2.1), we obtain

$$NT_n = GT_n + M_n \quad [4]$$

where NT_n is the nutritional target coordinate (i.e., optimal) value for n in nutrient space, GT_n is the coordinate for n of the growth target, and M_n is the amount of n needed to fuel metabolism when feeding on a nutritionally ideal food under optimal environmental conditions.

Thus, when feeding on a nutritionally and environmentally optimal food:

$I_n = IT_n$ (the coordinate level of n at the intake target)

$$(R_g + Rr + "Rs)_n = GT_n$$

$$("Dm + Dc + Rc + "Rp)_n = M_n$$

W_n will be minimized and this will be true simultaneously for all nutrients.

If the amount of n eaten (I_n) is less than that required to reach the nutritional target level, then the insect will not be able to reach the growth target coordinate for n , unless an excess of other nutrients can be converted to make good the deficiency. For instance, deamination of ingested amino acids may provide metabolic fuel or structural carbohydrate/lipid when amounts of carbohydrate eaten are low (Hinks et al., 1993; van Loon, 1988; Raubenheimer and Simpson, 1993). Nutrients are often not mutually interconvertible, however, as seen, for example, between carbohydrate and protein, or between amino acids within protein (e.g., see Dadd, 1985; van Loon, 1988).

Provided that all the ingested n is available for digestion, it would be expected that potential wastage (W_n) would be minimal when intake is less than or equal to requirements, where requirements are the combined sum of GT , M , and any extra energetic costs associated with obtaining and processing suboptimal diets [Rabenheimer and Simpson (1994); see also Karowe and Martin (1993), Martin and Van't Hof (1988), and Slansky (1993) for detailed discussion of such potential costs]. On the other hand, if intake exceeds requirements, then there will be wastage. Because, by definition, there are fitness costs associated with exceeding the nutritional target (just as there are for not reaching it), it would be expected that amounts ingested above requirements should be removed from the insect or converted for use elsewhere. Fitness costs may stem from an excess of a particular nutrient being toxic, or else jamming the feeding control systems (Section 9.3.4) and thereby preventing intake of sufficient amounts of other nutrients [i.e., cause incidentally restricted intake of others, *sensu* Rabenheimer (1992)]. Other ecological consequences of overeating may also reduce fitness.

9.4.2. Utilization Plots

The efficiency with which nutrients are processed may be clearly visualized in bicoordinate plots of uptake (intake or absorption) and the various components

of nutrient budgets [termed "utilization plots" by Raubenheimer and Simpson (1994)]. Such plots are the geometrical representation of analysis of covariance (ANCOVA) designs. ANCOVA provides a more statistically powerful and valid means of analyzing such data than do the widely used nutritional indices (Waldbauer, 1968), with which there are not only statistical (Raubenheimer and Simpson, 1994), but also potentially severe methodological problems (Farrar et al., 1989; van Loon, 1991, 1993; Schmidt and Reese, 1986; Simpson and Simpson, 1990).

A hypothetical utilization plot is shown in Fig. 9.9. It indicates the pattern of utilization for a given nutrient which would be expected across a range of intakes. For intakes below requirements, wastage is close to zero, while ingested nutrient above this level is removed from the animal (see equation [2]).

Patterns very similar to this were found for utilization of ingested nitrogen and carbohydrate in experiments on nymphs of *L. migratoria* (Zanotto et al., 1993; Fig. 9.10). Locust nymphs removed nitrogen in excess of needs principally postabsorptively, by increasing uric acid production (i.e., Dex_n in equation [2]). Catabolism and oxidation of excess amino acids have also been reported in larval

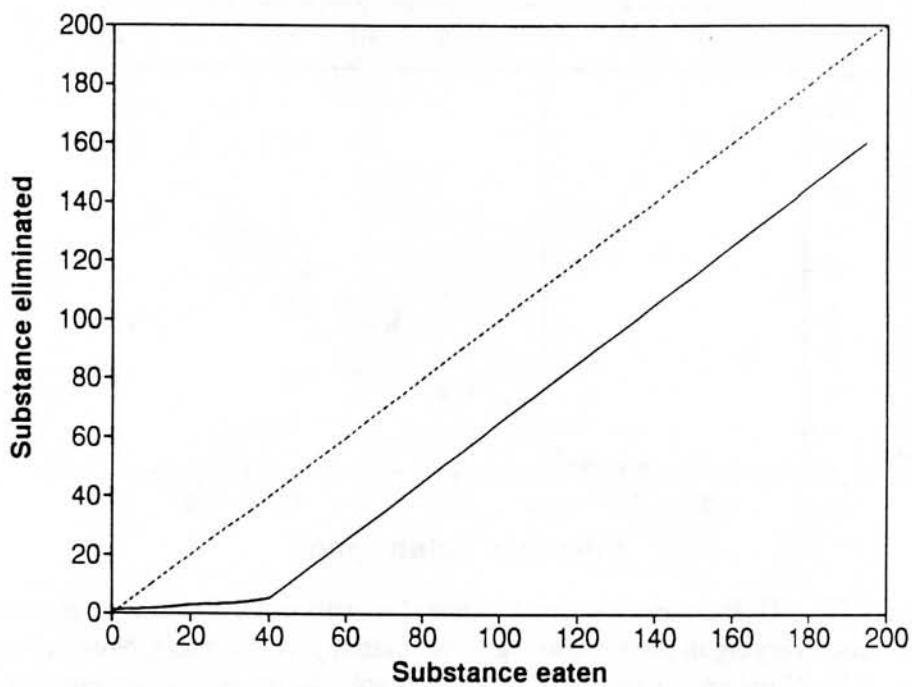


Figure 9.9. A hypothetical utilization plot for a nutrient. The dotted line represents the expected pattern for a non-nutrient (all that is eaten is eliminated as wastage, with none being used for maintenance, behavior, or growth). The solid line shows the case where virtually all of a nutrient ingested is used up to the level required for maintenance, growth, and behavior. Intake beyond this level (40 arbitrary units on the figure) is eliminated from the animal (by egestion, excretion, or wastage respiration). (Adapted from Raubenheimer and Simpson, 1994.)

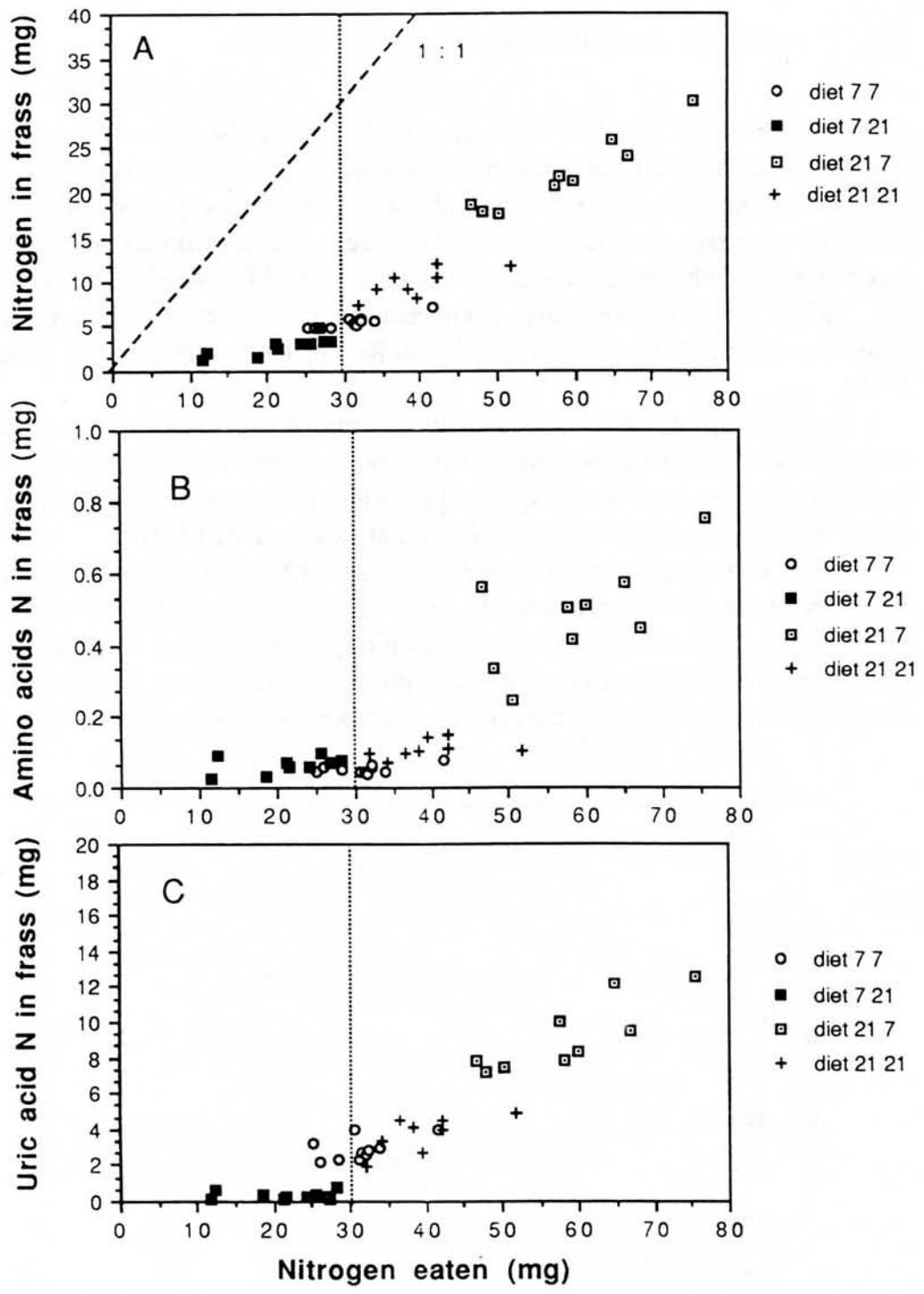


Figure 9.10. Utilization plots for nitrogen for fifth-instar *Locusta migratoria* fed one of four foods varying in protein and digestible carbohydrate content. Note the resemblance to Fig. 9.9. Numbers to the right of each graph show percent protein and digestible carbohydrate in the diets. (A) Graph showing the total nitrogen in the frass. When the insects consumed less than 30 mg of nitrogen, relatively little was excreted/egested, whereas when intake exceeded 30 mg, the rate of removal was much higher. (B) Graph indicating the amount of free amino acids in the frass. A similar pattern to total nitrogen is evident, although amino acids contributed only a small proportion of the total. (C) Graph showing uric acid excreted. When consumption of nitrogen exceeded 30 mg, uric acid excretion was a major component of total nitrogen removal. Notice differences in the scales of y-axes between graphs A and C. (After Zanotto et al., 1993.)

Bombyx mori (Horie and Watanabe, 1983), *Pieris brassicae* (van Loon, 1988) and *Spodoptera eridania* (Karowe and Martin, 1989).

Within the nitrogen budget for locust nymphs there was evidence for selective removal of lysine (Zanotto et al., 1994). This is the most effective of the suite of amino acids whose combined presence is required in the hemolymph at elevated concentrations to cause reduction in behavioral and gustatory responsiveness to amino acids in the food (Simpson et al., 1990a; Section 9.3.4). Selective removal of lysine would be expected to alleviate inhibition of feeding for more limiting nutrients. The site of control of lysine removal is unknown but could occur via low rates of absorption from the gut (Deu_n in equation [2]), high rates of excretion, and/or low rates of resorption from the rectum (either of the last two increasing Dex_n in equation [2]).

In locust nymphs, excess carbohydrate was removed mainly by wastage respiration (" Dm_n in equation [2]) (Zanotto et al., 1993), as has been suggested to occur in larval *Samea multiplicalis* (Taylor, 1989) and is known to occur through the activities of brown adipose tissue in vertebrates (Rothwell and Stock, 1979).

Although possible, there is little or no evidence as yet in insects for preabsorptive removal of excess nutrients (Deu_n and Ded_n in equation [2]) through, for instance, increasing gut emptying rate (without concurrently decreasing intermeal intervals), declining enzyme:substrate ratio (due to there being a fixed or decreasing rate of digestive enzyme secretion), or lowered absorption rates across the gut.

Gut emptying rate either does not change (Simpson and Abisgold, 1985) or varies negatively with the concentration of nutrients in ingested food (e.g., Yang and Joern, 1994a), in part as a result of hormonal and osmotic effects on gut motility (e.g., Cazal, 1969; Gelperin, 1966). These effects on intrinsic rate of emptying are enhanced by increased consumption of nutritionally dilute foods (this being driven by the more rapidly emptying gut and low hemolymph nutrient titers; see Section 9.3), so that incoming food physically pushes existing food backwards in the gut, further shortening residence time. Increased consumption of nutritionally dilute foods has also been shown to be accompanied by a rapidly invoked increase in gut size (Yang and Joern, 1994b), which might increase digestive and absorptive efficiencies.

Levels secreted of digestive enzymes either do not vary with dietary nutrients or are positively correlated [Broadway and Duffey, 1986; Lemos et al., 1992 (for trypsin)], although there is a report of lowered production of aminopeptidase in larvae of *Ceratitis capitata* fed high- versus low-protein foods (Lemos et al., 1992). In theory, egestion of excess nutrient will occur if only enough digestive enzymes are produced to provide the tissues with required levels of a nutrient; any ingested nutrients above this required level will pass undigested through the gut (Raubenheimer and Simpson, 1993). At least in locust nymphs this was found not to be the case for both protein and carbohydrate (Zanotto et al., 1993), where virtually all ingested nutrients were digested and absorbed over a wide

range of intakes. This is perhaps not surprising, given that digestive enzymes hydrolyze macromolecules into their constituents; and it is these constituents (e.g., amino acids, monosaccharides), not the dietary macromolecules (whose composition is variable), whose levels must be regulated in the body. Similarly, the digestive yield from a given titer of enzyme will vary with, for instance, gut passage time, temperature, and other dietary constituents (Karowe and Martin, 1993; Slansky, 1992, 1993; Yang and Joern, 1994a).

Such regulation could occur via altered rates of absorption of nutrients from the gut. Changes in concentration gradient between the gut lumen and hemolymph might influence uptake of nutrients which are absorbed passively, while active absorption processes found for other nutrients might also vary (Dow, 1986; Turunen, 1985; see also Kasarov and Diamond, 1988). Evidence is lacking for such effects in insects, however.

9.5. Summary

The multidimensional geometric approach to insect feeding and nutrition has provided a framework for exploring and uniting mechanistic, functional, ontogenetic, and evolutionary questions (Simpson and Raubenheimer, 1993a). The present review has highlighted the mechanistic aspect of the framework.

Meeting nutritional needs (approaching the nutritional target) involves regulation of feeding and also postdigestive utilization of food. Regulating intake (reaching the intake target or point of best compromise) requires that the animal integrate information about the nutritional quality of its food and its own current nutritional state. The former information is provided by sensory inputs whose behavioral efficacy is to some extent genetically hard-wired, but is also modulated by previous nutritional experience. Information regarding nutritional state comes in large part from the hemolymph and is linked to foraging, dietary selection, and control of meal size through various mechanisms, including direct modulation of taste receptor responsiveness and learning. Such multiple mechanisms provide the basis for considerable behavioral flexibility.

Insects can move from their point of intake in nutrient space to approach their growth target by differentially utilizing nutrients postdigestively. Such postdigestive compensation can involve a range of responses and is usefully displayed by "utilization plots," which are the graphical representations of analysis of covariance designs.

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