AN INTEGRATED APPROACH TO BAITING STRATEGIES FOR THE GERMAN COCKROACH, *BLATTELLA GERMANICA* (L.) (DICTYOPTERA: BLATTELLIDAE)

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Abstract - Though much is known about cockroach baiting systems, that knowledge is highly compart-mentalised, reducing its utility. We advocate an integrated approach to the study of cockroach baiting, which focuses on the inter-actions among various food components, and readily integrates across behavioural, physiological and developmental levels of analysis. An experiment planned and analysed within an intergrative framework (the 'Geometric Framework') is described, demonstrating the manner in which protein and carbohydrate interact in the regulation of feeding by Blattella germanica nymphs. It also demomnstrated the interactions among behavioural, physiological and developmental means of regulation. Protein status was primarily regulated through differential rates of ingestion, whereas carbohydrate was primarily regulated by post-ingestive means. Some implications for bait strategies are discussed, as well as the extension of the integrated approach to include aspects of cockroach biology beyond food composition.
Key words - Geometric framework, nutrition, control

INTRODUCTION

There are many empirical data concerning cockroach baiting in the literature, both about outcomes which directly indicate baiting success (e.g. changes in population size: Zeman *et al.*, 1992) and about underlying mechanisms (e.g. diet consumption rates: Cooper and Schal, 1992; Cochran, 1983; Gordon, 1968; Haydak, 1953; insecticide metabolism rates: Siegfried *et al.*, 1990; foraging behaviours: Kanayama *et al.*, 1993; Cloarec and Rivault, 1991; Silverman, 1986; DeMark *et al.*, 1993; insecticide taste: Strong *et al.*, 1993). These data have identified mechanisms which significantly affect baiting success, but have not as yet been sufficient to allow quantitative predictions of the outcomes of baiting strategies. This is not primarily because the wrong mechanisms have been investigated but because, at present, their descriptions exist in isolation, whereas the dynamics of baiting systems are the result of the simultaneous effects of multiple mechanisms and their interactions. Therefore, no single factor, however important, can be the sole determinant of nutritional behaviour and various mechanisms must be considered simultaneously (Figure 1). It is our belief that some focus on the integration of various causal factors will lead to improved baiting strategies.

We wish to emphasise the distinction between our use of the term 'integration' and another context in which it is used in pest management, namely in 'integrated pest management'. This phrase is used to denote strategies that concurrently manage several pest species and their predators, using a diversity of control methods (Levins, 1986). By contrast, we use the term 'integration' to describe studies whose explicit focus is on the interactions among the numerous causal factors underlying the behaviour of biological systems (Raubenheimer and Simpson, 1999).

Our aim in this paper is to present and illustrate, using data for cockroaches, a geometrical approach to the integration of nutritional studies (reviewed in Simspon *et al.*, 1995; Raubenheimer and Simpson, 1997). We discuss some implications of the approach for baiting strategies, and consider its extension to cover relevant aspects of baiting beyond the interaction of the organism and food components.

MATERIALS AND METHODS

Geometric framework

Most of our knowledge about nutritional behaviour concerns each nutrient in isolation (Fig 1a). This experiment was planned within the 'Geometric Framework'. This framework allows the interactive effects of any number of nutrients to be observed (Fig. 1b), and to be interpreted in behavioural, physiological and developmental terms. Its detail and use have been described elsewhere (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993), so we present only a brief description here.

The nutrient requirement of an animal is depicted as a point in n-dimensional hyperspace, subtended by axes representing the nutrients of interest. An animal may reach this point, which we call the 'intake target', by eating either a nutritionally balanced food or by switching among two or more imbalanced but complementary foods. For example, in Figure 2 (which represents just two nutrient dimensions: protein and carbohydrate) the animal may reach the intake target by feeding either on food A, or on a mixture of complementary foods, B and C, which jointly make the shaded area accessible to the animal.

Foods

In terms of the regulation of food intake, protein and carbohydrate are the two nutrient types of greatest importance to most animals. Dealing quantitatively with these nutrients requires that experimental foods be of known nutrient content. We have therefore used synthetic foods whose composition can be tightly controlled. The foods were made according to Simpson and Abisgold (1985), then powdered and reconstituted with 1g agar per 10 g food, dissolved in ~3 ml of water. Foods comprised either protein or carbohydrate at either 30% or 60% w/w and were identical in the concentration of all other nutrients, except amounts of cellulose (Table 1). Nymphs were allowed to select their diet from a choice of two foods. One food contained protein, but not soluble carbohydrates and the other food contained soluble carbohydrates, but not protein.

Animals

Blattella germanica nymphs of both sexes and weighing over 20 mg were taken from the lab culture and housed individually in clear perspex boxes (40 x 113 x 170 mm). No harborage was provided. The insects were maintained on the culture foods (rabbit pellets and potato) and inspected daily. Those that had moulted and showed pronounced wing buds were taken to have entered the last nymphal instar. In each treatment, nymphs were given either both concentrated foods, both dilute foods, or one of each (Table 2). Water was available from an open dish. Insects were assigned to treatments according to a randomised block design. Every two days the insects were inspected and the amounts of food eaten and faeces produced were weighed. The insects were weighed at the start and at the end of the stadium. They were frozen after the imaginal moult and the carcasses were subjected to lipid extraction with chloroform (Loveridge, 1973) and to micro-Kjeldahl analysis for total nitrogen. A conversion factor of 6.25 (Holme and Peck, 1993) was used to estimate total protein content.

Insects absorb protein and soluble carbohydrate with close to 100% efficiency (Zanotto *et al.*, 1993), such that their faeces consist almost entirely of non-digested bulk cellulose and agar. Therefore, if the mass of faeces produced is less than the amount of bulk ingested over a stadium, the difference may be taken as a conservative estimate of the amount of cellulose digested (any nutrients in the faeces will reduce the estimated amount of cellulose digested). We calculated the amount of bulk ingested by each insect and used it to estimate the amount of cellulose digested.

Data analysis

The data was plotted in the two nutrient dimensions of interest – protein and carbohydrate. ANCOVA analysis was used to determine significant predictors of amounts of nutrients stored, ingested and digested. Significant predictors were used to build best-fit general linear models. Kaplan-Meier was used as a failure-time analysis for the emergence data (Fox, 1993).

RESULTS

Treatment differences in growth

The amount of nitrogen in the carcasses after imaginal moult was affected by sex and weight at the start of the stadium, but there was no significant effect of treatment (Table 3). In contrast, the amount of fat in the carcasses was affected by sex, start weight and treatment (Table 3). Therefore, protein derived growth was regulated tightly around a 'growth target' (Raubenheimer and Simpson, 1993) in the face of perturbations in food composition. Attainment of the carbohydrate target was less robust.

Table 1. Food constituents (g per 100g of food)

Nutrient	60% protein food	30% protein food	60% carbohydrate food	30% carbohydrate food
Casein	30	15	0	0
Peptone	15	7.5	0	0
Albumin	15	7.5	0	0
Sucrose	0	0	30	15
Dextrin	0	0	30	15
Cellulose	26.9	56.9	26.9	56.9
Agar	9.1	9.1	9.1	9.1
Salts and vitamins	4	4	4	4

Table 2. Combinations of foods used in the four treatments

Treatment	*Protein food*	*Carbohydrate food*
1	60%	60%
2	30%	30%
3	60%	30%
4	30%	60%

Table 3. ANCOVA F-values and adjusted r^2 values from best fit linear models for amounts of nutrients ingested. (a) not allowing for 'protein ingested' as a predictor. (b) allowing 'protein ingested' as a predictor.

Predictors	Carcass nitrogen weight	Carcass lipid weight	Protein ingested	Carbohydrate ingested[a]	Carbohydrate ingested[b]	Cellulose digested
sex	198.083***	9.434***	144.327***	32.885***	3.75	29.298***
start weight	20.928***	6.628*	-	7.312**	4.10*	-
treatment	-	23.675***	10.650**	81.462***	26.18***	11.563***
protein ingested					9.73***	
sex*start weight	-	-	-	-	3.90*	-
sex*treatment	-	-	-	3.550*	4.22***	-
Adjusted r^2	0.848	0.544	0.697	0.803	0.844	0.465

Behavioural compensatory responses

Both sex and treatment affected the amounts of both protein and carbohydrate ingested (Table 3). Despite the treatment effects, Figure 3 shows that amount of protein ingested was, in fact, tightly regulated. Insects with the 30% protein food (treatments 2 and 3) ingested considerably more than half of that ingested by insects with the 60% food (treatments 1 and 4) over the stadium. The 'intake target' for protein was thus well defended despite food dilution. Figure 3 also shows that carbohydrate was less well regulated, with the amounts of nutrient ingested on 30% carbohydrate foods (treatments 2 and 4) being much lower than on 60% carbohydrate foods (treatments 1 and 3).

   A distinct temporal pattern of ingestion was seen for protein and carbohydrate, for both males and females and for all 4 treatments. Protein consumption was maximal at the start of the stadium, and decreased throughout. Carbohydrate consumption was maximal on day 4. The ratio of ingested protein: carbohydrate was therefore seen to vary through the stadium. The total amount of carbohydrate ingested was significantly predicted by the total amount of protein ingested (Table 3).
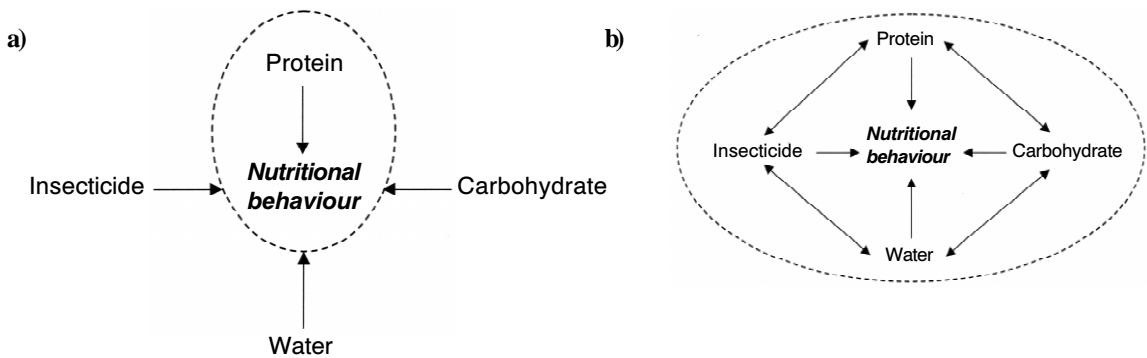

Figure 1. (a) Representation of the usual approach to nutritional systems, in which the effects of each dietary constituent are independently investigated. This has shown which constituents significantly affect the system, but does not reflect the nature of the system. (b) Representation of an integrated approach, in which the simultaneous effects of several food constituents are studied. This reflects the biology of a nutritional system, in which nutrients are known to have interactive effects (some shown).
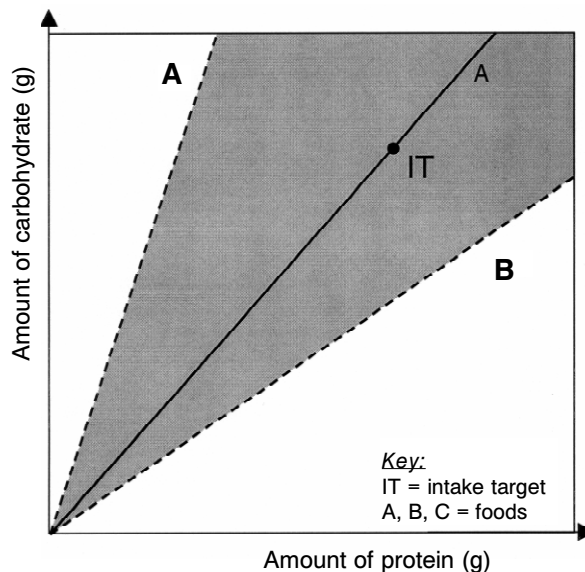

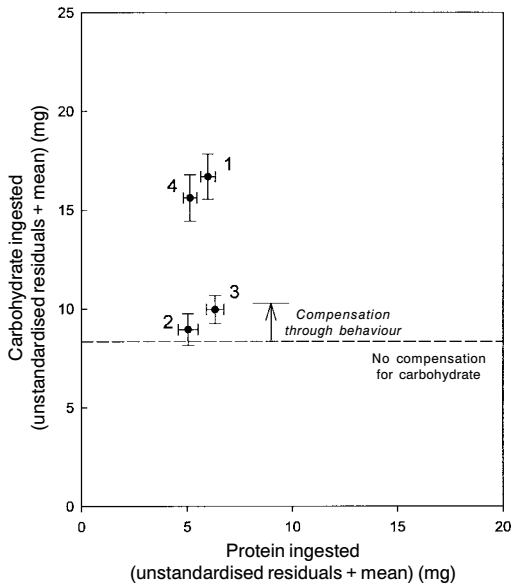**Figure 2.** The Geometric Framework: in two nutrient dimensions (see text for explanation)

Figure 3. Nutrient ingestion in the Geometric Framework: Amounts of 'protein ingested' (mg) and 'carbohydrate ingested' (mg) through the stadium, presented as treatment means plus unstandardised residuals of General Linear Models each with 'sex' as sole predictor, with 95% confidence intervals. Numbers refer to treatments (see Table 2).
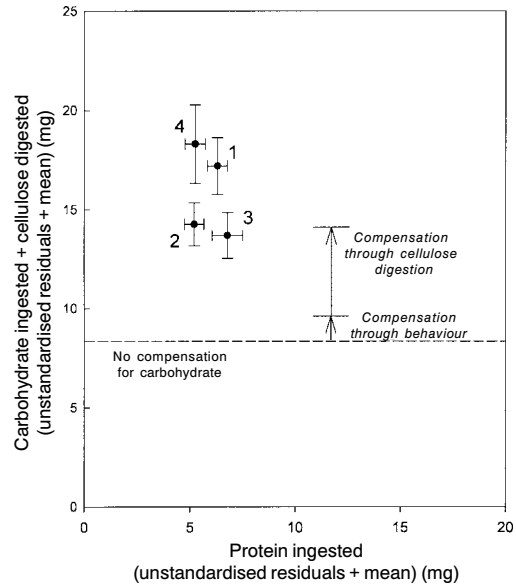
Figure 4. Nutrient digestion in the Geometric Framework: Amounts of 'protein ingested' (mg) and 'carbohydrate ingested plus cellulose digested' (mg) through the stadium, presented as treatment means plus unstandardised residuals of General Linear Models each with 'sex' as sole predictor, with 95% confidence intervals. Numbers refer to treatments (see Table 2).
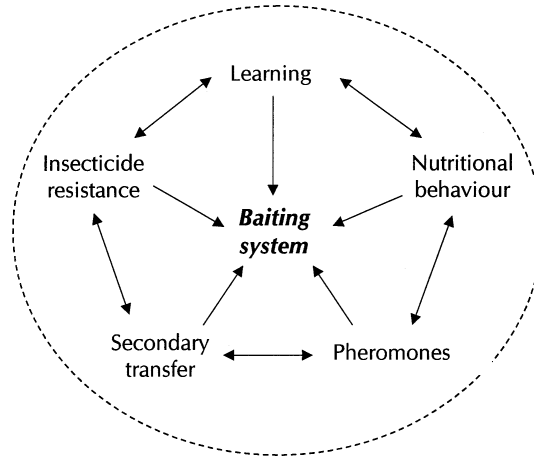

Figure 5. Representation of an integrated approach to baiting systems.

Physiological compensatory responses

Significant differences between treatments were seen in the amount of cellulose digested (Table 3), increasing in the order: both foods concentrated, protein food diluted, carbohydrate food diluted, both foods diluted. Increases in cellulose digestion had the effect of increasing available glucose on dilute foods. Therefore, digested cellulose can be put on the same nutrient axis as carbohydrate ingested (Figure 4). This shows that cellulose digestion reduced differences in available carbohydrate and so functioned as a means of regulating carbohydrate status. A two-way ANOVA showed that both protein and carbohydrate concentrations significantly affected the amount of cellulose digested (protein: $p=0.001$, carbohydrate: $p<0.001$), but that there was no significant interaction term.

Developmental compensatory responses

Instar duration was influenced by treatment (Kaplan-Meier: sex p=0.013, treatment p=0.029), being longer for insects presented with both dilute foods. Since the pre-moult aestivation duration was constant across treatments, there was an increased feeding time duration for insects given both dilute foods. That is, the insects compensated for slow movement along their selected nutrient trajectory (which was imposed by limits on the rates of nutrient assimilation) by extending its duration.

DISCUSSION

Our data show the significance of interactions between nutrients in the nutritional behaviour of *B. germanica* nymphs. Sixth instar nymphs were seen to regulate their nutritional status, compensating for macronutrient dilution and balance. The outcome of this regulation, which was more accurate for nitrogen than for carbohydrate, was to store a fixed mass of nutrients by the start of adulthood,. It was achieved at a number of levels (behaviour, physiology, development) and, at each level, an interaction between nutrient types was seen.

At the behavioural level, the amounts of foods ingested varied between treatments, with nymphs 'defending' an ingestion target. This was tightly defended for protein, but not for carbohydrate. Further, the amount of carbohydrate ingested was significantly affected by the amount of protein eaten, showing an interactive effect of these macronutrients on feeding behaviour.

At the physiological level, our results indicate that B. germanica nymphs are capable of digesting cellulose, but that the extent to which they do so is affected by the nutrient content of their foods. Wharton and Wharton (1965) found evidence for cellulase production in all 20 species of cockroach studied, except *B. germanica*. The discrepancy between our result and that of Wharton and Wharton (1965) may therefore be due to the foods available to their insects, the details of which they did not publish.

The digestion of cellulose functioned as a means of regulating carbohydrate status. Figures 3 and 4 show how much carbohydrate was ingested and digested, respectively, on each treatment, with dotted reference lines indicating the amounts that would have been ingested/digested if no compensation for dilution had occurred. A comparison of the figures shows that most of the compensation for carbohydrate was achieved, not through increased ingestion of dilute foods (dilute carbohydrate foods, treatments 2 and 3, are barely above the line of 'no compensation' in Fig. 3), but through increased digestion of cellulose (Fig. 4).

Adults of *Periplaneta americana* only digest significant amounts of cellulose when both fed high protein foods and nutritionally stressed by the removal of their endosymbionts (Mira, 1999). Our results show that, in *B. germanica* nymphs, the amount of cellulose digested was increased in response to protein dilution. Insects on dilute protein foods ingested less carbohydrate than those on concentrated protein foods (Fig. 3), but were seen to digest more carbohydrate once cellulose digestion was included (Fig. 4). This suggests that insects on concentrated protein foods deaminate amino acids and use them in carbohydrate metabolism, with nymphs on dilute protein foods digesting more cellulose as a means of regulating carbohydrate status.

At the developmental level, our data showed a developmental response to food content in nymphs. The dilution of one nutrient type alone (either protein or carbohydrate) has no effect on stadium duration, but when both foods were diluted, the duration of the stadium increased. Schal *et al.* (1993) have shown protein ingestion to affect juvenile hormone synthesis in *B. germanica* adult females, and Osorio *et al.* (1998) argue that nutrient intake affects the activity of the corpora allata, which is involved in the regulation of the ovarian cycle. This effect on the moulting cycle may be achieved through nutrient feedbacks acting upon hormones, as for the ovarian cycle.

This study has several specific implications for baiting strategies. Firstly, we infer that feeding behaviour in *B. germanica* nymphs is directed primarily to the end of regulating protein intake, and that the

ingestion of carbohydrate is less well regulated, with compensation for carbohydrate occurring mostly through post-ingestive mechanisms (when foods contain cellulose). Therefore, a high-protein bait will be highly acceptable to nymphs that have not yet reached their protein requirement, but aversive to those who have done so.

This prediction is complicated by the fact that a given intake rate may be achieved by regulating meal size and/or meal frequency. These together define meal patterns (Simpson *et al.*, 1995), which have complex implications for baiting systems. For example, small meals may lead to decreased kill rates through increased food-aversion learning, whilst large meals may only be taken very rarely or by just a fraction of the population. Therefore meal patterns must also be understood before long term measures can be used to make control recommendations. We have preliminary data suggesting that there is no effect of food content on meal frequency in cockroach nymphs and adult males, but that the relative acceptance of foods and the sizes of meals taken vary in response to food nutrient content. Further observations of feeding patterns are required before the data we present above may be utilised. We are currently engaged in making such observations.

Secondly, it has been suggested that providing the nutrients lacking in the environment may lead to greater bait uptake (Kells and Bennett, 1998). Our results suggest, however, that with resistant populations, it is also likely to significantly increase population growth rates.

These instances of the complex dynamics of nutritional behaviour illustrate the requirement for an approach that models the interactions among causal factors. This approach is, of course, applicable to other aspects of whole organism biology (Figure 5). It is therefore our aim to extend this approach to other factors which have been shown to, or are expected to, affect the success of cockroach baiting. These include foraging patterns (Cloarec and Rivault, 1991), trail pheromones (Krivosheina and Shatov, 1995; Brousse-Gaury, 1975), secondary toxin transfer (Kopanic and Shal, 1997), insecticide resistance (Hemingway and Small, 1993) and learning (Raubenheimer and Tucker, 1997). Whilst each of these factors has a significant isolated effect, significant interactions are also likely to occur. For example; resistance will greatly affect the opportunity to show food-aversion learning; trail pheromones will interact with individual foraging patterns.

The end goal is therefore an integrated model which aids in assimilating our knowledge of each of these factors and their interactions, and thus in predicting the control outcomes of proposed baiting strategies. Such a model ought greatly to assist the rational planning of baiting strategies. Its development would be greatly aided by collaborative experiments planned in an integrated framework.

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