Constraints on nutritional compensation in acridids

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Thesis submitted for the degree of Doctor of Philosophy

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ABSTRACT

Some aspects of the ability of locusts and grasshoppers (Acrididae) to compensate for nutritional shortfalls were studied, with a special emphasis on the factors which constrain this ability.

Chapter 1 investigates the effects over the short-term (12 h) of the plant-produced allelochemical tannic acid on the ability of *Locusta migratoria* (L.) and *Schistocerca gregaria* (Forskal) to compensate for dilution of dietary proteins and carbohydrates by increasing consumption. Tannic acid had no effect on compensatory feeding by *L. migratoria*, and stimulated feeding by *S. gregaria*.

Chapter 2 extends this study over the longer-term (fifth instar) for *L. migratoria*. Over this period, tannic acid restricted intake and reduced growth of those insects fed low-protein diets, indicating an inhibitory effect on compensatory feeding for protein. In addition, the levels of dietary proteins influenced regulation for carbohydrate intake and, to a lesser extent, vice-versa. A detailed discussion is presented of the ways that some dietary components can influence the intake of others, and how failure to take this into account can lead to poor experimental design and interpretation.

Chapter 3 investigates some mechanisms involved in dietary selection by the grasshopper *Schistocerca americana* (Drury). It was found that *S. americana* conditioned on distinctly flavoured protein-inadequate diets then tested on nutritionally similar diets with the familiar or a novel flavour, tend to eat more of the novel-flavoured diets. This suggests that conditioned neophilia, possibly in conjunction with aversion learning, may be a factor facilitating dietary selection in acridids.

Chapter 4 investigates the patterns of feeding and dietary selection behaviour of the polyphagous grasshopper *Taeniopoda eques* (Burmeister) in its natural desert habitat. Despite the overwhelming thermoregulatory requirements and unpredictable variability inherent in ecological complexity, these insects nonetheless maintained a pattern of feeding comparable to that observed under controlled laboratory conditions. The patterns of dietary selection behaviour were concordant with some of the mechanisms observed to operate in the laboratory.

Chapter 5 addresses an important inadequacy in the methodology currently used to investigate some aspects of nutritional compensation. A computer-generated data set is used to illustrate how the analysis of the currently popular ratio-based nutritional indices may be flawed, and how this may be overcome using as an alternative the analysis of covariance.

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GENERAL INTRODUCTION

The tissues of plants and animals differ widely in their chemical composition, and this poses certain nutritional "hurdles" (Southwood 1973) for herbivorous insects. At the coarse-grained, evolutionary scale these problems are clearly not insurmountable, as evidenced by the spectacular diversity and abundance of phytophagous insects. The solutions include a wide range of behavioural, physiological and morphological adaptations which effectively bridge the gap between the nutritional requirements of herbivorous insects and the chemical composition of their host plants.

At the more fine-grained level of the individual insect, however, there are further problems. The chemical composition of plants is highly variable, both between species and within species across space and time (Chapman 1990). Added to this is the fact that the nutritional requirements of insects change in time and with different environmental conditions (Bernays and Simpson 1990, Simpson and Simpson 1990). The gap between nutritional requirements and availability is thus in a continual state of flux, and it might therefore be expected that herbivorous insects would have homeostatic mechanisms enabling them to respond to the resulting uncertainty in a flexible and adaptive way. These homeostatic, or compensatory mechanisms have in recent years been the subject of increasing focus in the study of plant/insect interactions.

There are two well established categories of compensatory response. First, an insect may satisfy its nutritional requirements by feeding eclectically from a variety of foods. This, recently reviewed by Simpson and Simpson (1990) and Waldbauer and Friedman (1991), has been termed *dietary self-selection*. Secondly, an insect may continue to feed on the present food, but adjust the rate of consumption so as to increase the amount of any limiting nutrients ingested (known as *compensatory feeding*; reviewed by Simpson and Simpson 1990). There are numerous examples of both phenomena in the literature, and the regulatory mechanisms have in some cases been elucidated (Simpson and Simpson 1990). A third possibility, but by no means as well established, is that insects may compensate post-ingestively by altering the efficiency with which nutrients are digested, absorbed or metabolised. While there are several apparent examples, it remains to be demonstrated that this is an active compensatory response by the insects and not merely a passive outcome of experimental and analytical protocols (further discussed in Chapters 2 and 5).

The aim of the present study is to investigate some of the factors which may constrain nutritional compensation in the Acrididae. This has, despite the increasing attention paid in recent years to the subject of nutritional compensation, remained a largely unresearched area and, with the exception of that due to Simpson and Simpson (1990), has received little explicit discussion. The acridids are

particularly well suited to such a study, considering that more is known about food selection (Chapman 1990) and factors regulating intake (Simpson 1990) in these than any other insects.

Chapters 1 and 2 deal with the constraints on compensatory feeding due to the interactions which take place between coingested compounds. In chapter 1, the topic is introduced with a detailed study of the short-term (12 h) effects of the plant-produced allelochemical tannic acid on compensatory feeding by nymphs of *Locusta migratoria* (L.) and *Schistocerca gregaria* (Forskal). The presence of tannic acid in the diets resulted in increased consumption by *S. gregaria*, and had no effects on the ability of *L. migratoria* to compensate for levels of dietary proteins and carbohydrates. In Chapter 2, this study is extended to the longer-term (throughout the fifth stadium) for *L. migratoria* and expanded to consider the simultaneous influence of dietary imbalance and tannic acid.

In Chapter 3, I turn my attention from compensatory feeding to the mechanisms involved in dietary self-selection. Self-selection can be constrained by behavioural phenomena such as foodplant induction, in which an insect shows a decreased tendency to switch from a plant on which it has fed for some time even if the alternative is nutritionally superior (Jermy et al. 1968, Jermy 1986). By contrast, mechanisms which increase the tendency of insects to feed on alternative plants following experience with

nutritionally suboptimal ones may facilitate self-selection (the so-called "novelty effect"). Chapter 3 describes a study into the selection by *Schistocerca americana* (Drury) nymphs of artificial diets with novel or familiar flavours, following pretreatment with flavoured diets differing in protein content.

The above studies, and indeed all previous studies into detailed aspects of feeding behaviour in insects, have been performed under laboratory conditions. This gives rise to important questions regarding the role in a more complex field situation of the mechanisms observed to underlie feeding behaviour in the laboratory. For example: How do these mechanisms interact with the unpredictable variability characteristic of ecological complexity? To what extent, if at all, can the patterns of feeding observed in the laboratory be quantified in a field situation? Are there additional, ecological constraints to nutritional compensation which cannot be observed in the laboratory? Chapter 4 describes an initial attempt at answering these questions, by recording in detail the feeding behaviour of the polyphagous grasshopper *Taeniopoda eques* (Burmeister) in its natural habitat in the Arizona desert. Individual insects were watched continuously for up to 690 minutes, and this enabled a detailed analysis of dietary breadth and feeding behaviour throughout the day.

Finally, in Chapter 5, I address a statistical aspect to the study of nutritional compensation. Nutritional studies -

particularly those investigating post-ingestive compensation where the phenomena of interest may be subtle changes in parameter values - rely on a high degree of experimental precision. It therefore goes without saying that the choice of analytical procedures for such data should be made with prudence. To date, much of the published data on quantitative aspects of insect nutrition are expressed in the form of the ratio-based nutritional indices proposed by Waldbauer (1968). Chapter 5 demonstrates how the statistical analysis of these indices may be flawed, often leading to biologically incorrect conclusions. A computer-generated data set is used to illustrate this, and how these incorrect conclusions may be avoided by using as an alternative the analysis of covariance.

Notes on thesis structure

All of the chapters presented here are at various stages of publication in different journals (see the title page to each chapter for details). The overall structure of each chapter (Abstract, Introduction, Methods, Results, Discussion, Acknowledgements and References) is as submitted, and the stylistic details of the respective journals have been retained. However, some minor changes have been made. Throughout, I have referred to chapter numbers where the work described in other chapters of this thesis is addressed. In Chapter 2, I have taken the liberty of extending the discussion beyond the length permissible

in most journals, including a more full discussion of some of the aspects referred to in the submitted version. No doubt, further changes will be made to those manuscripts currently being reviewed for publication. In addition to the various detailed chapters, there is a brief General Introduction and General Conclusions. Literature cited in these is listed in the General References.

Finally, this research was performed under the generous guidance, encouragement and, where necessary, assistance of my mentors Steve Simpson and Liz Bernays. In addition Liz Bernays provided funds for my stay in the USA. In accordance with the protocols of scientific publishing, authorship of the published/submitted versions is therefore shared. Chapters 3 and 4, both researched while visiting Liz Bernays' laboratory at the University of Arizona, have been submitted under the authorship of Bernays & Raubenheimer and Raubenheimer & Bernays, respectively. Chapter 1 has been published, and 5 submitted for publication under the authorship of Raubenheimer & Simpson. For jointly authored publications, I have changed the singular "I" appearing in this thesis to the plural "we".

Chapter 1

The effects of simultaneous variation in protein, digestible carbohydrate and tannic acid on the feeding behaviour of larval *Locusta migratoria* (L.) and *Schistocerca gregaria* (Forskal) I: short-term studies

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ABSTRACT. The influence of simultaneously varying the levels in artificial diets of protein, digestible carbohydrate (14% or 28%) and tannic acid (absent or 10%) on the feeding behaviour of the oligophagous *Locusta migratoria* (L.) and the polyphagous *Schistocerca gregaria* (Forskal) (Acrididae) was investigated. Total consumption and detailed feeding behaviour were recorded over a 12-h period in choice and no-choice experiments. In addition, amounts eaten by *Schistocerca* of the 14% protein, 14% carbohydrate diet with and without tannic acid were measured at regular intervals throughout the fifth stadium, and insect growth over this period was recorded. There were no interactive effects of nutrient levels and tannic acid, despite the fact that both species compensated for dilution of dietary protein by increasing consumption. Only male *Locusta* compensated for dilution of dietary carbohydrates, and this compensation was much less marked than for protein. Tannic acid did influence feeding as a main effect, however. It caused an increase in amounts eaten by *Schistocerca* in both choice and no-choice experiments. This increased consumption was due to an increase in the number of meals taken. A shorter latency period before and a longer duration of the first meal by naive insects suggested a phagostimulatory rather than a post-ingestive effect of tannic acid. The stimulatory effect was only apparent for the first 24 h of continuous exposure, but this temporary enhancement none the less resulted in the

insects being heavier at adult ecdysis. Stadium duration was also somewhat reduced. In a no-choice situation, no effect of tannic acid on the feeding behaviour of *Locusta* was observed. When given a choice however, this species took significantly more meals on the tannic acid-free diet, these being of similar average size to meals taken on the tannic acid diet. Significantly more insects took their first meals on the tannic acid-free diet in the choice test, indicating a deterrent effect of tannic acid in *Locusta*.

Introduction

There have been numerous studies into the presumed defensive role of plant-produced tannins against insect herbivores, yet any general conclusions have so far eluded us (Bernays, 1981). This is partially due to the structural diversity and complexity of these compounds (Zucker, 1983), and to the inherent difficulties of studying the effects of plant-produced chemicals on herbivorous insects.

One such difficulty arises from the simultaneous needs of the experimenter to minimise uncontrolled variability due to the chemical complexity of plant tissue (eg., see Risch, 1985), while avoiding the dangers of over-simplification by taking into account possible interactive effects between chemicals. Such interactions include the intake of a particular nutrient being limited by an insect's

ability to cope with other (nutrient or non-nutrient) chemicals occurring in the food. For instance, many phytophagous insects are known to compensate for dilution of dietary nutrients by increased consumption (Simpson & Abisgold, 1985; Simpson & Simpson, 1990). A consequence of eating more of a food to ingest sufficient of a limiting nutrient is that more of any potentially deleterious compounds present are also ingested. This should manifest itself as an interaction term in any analysis of an experiment in which levels of dietary nutrients are reduced in the presence of a deleterious allelochemical.

A second difficulty is the proper interpretation of the insects' response to a given diet. Thus, an insect might eat more of one diet than another either because the former is more phagostimulatory (or less deterrent), or because it is nutritionally inferior and to provide the required level of nutrition more of it needs to be consumed (Simpson & Simpson, 1990). Similarly, diminished performance resulting from the ingestion of an allelochemical could be due to deterrence, toxicity, or both (Blau et al., 1978; Bernays & Chapman, 1986; Cottee et al., 1988). Deterrence could be due to an innate response of an insect to the sensory properties of food containing allelochemicals or due to post-ingestive effects of eating the diet (Lee & Bernays, 1988).

The study of insect nutrition has come some way in recent

years towards approaching these problems of the chemical complexity of plants, possible interactive effects between nutrients and the interpretation of the insects' response to diets varying in nutrient composition. This progress has been in part due to the introduction of techniques combining precise manipulation of chemicals in artificial diets with detailed observations of the patterns of feeding (Simpson & Abisgold, 1985; Simpson et al., 1988, 1989; Simpson, 1990). While a few studies have recently investigated possible interactive effects on insect herbivores between nutrients and allelochemicals (Lincoln et al., 1982; Hare, 1987; Broadway & Duffey, 1988; Johnson & Bentley, 1988; Slansky & Wheeler, 1989), none have combined this with detailed behavioural studies to elucidate the mechanisms underlying the insects' response. One purpose of this paper is therefore to introduce to the study of plant/insect interactions the technique of detailed behavioural analysis combined with the simultaneous variation of nutrients and allelochemicals in chemically defined diets.

This is the first in a series of papers in which I report on the detailed behaviour of two acridids, *Locusta migratoria* (L.) and *Schistocerca gregaria* (Forskal), in response to simultaneous variation in the levels of protein (14% or 28%), digestible carbohydrate (14% or 28%) and the hydrolysable tannin, tannic acid (10% or absent). *Locusta migratoria* is oligophagous, feeding only on grasses, in

which hydrolysable tannins do not occur, while the polyphagous *S. gregaria* feeds on a wide range of dicotyledonous plants known to contain these compounds. This leads to the expectation that the two species should respond differently to tannins, and previous studies have shown that *S. gregaria* is indeed tolerant of these compounds while *L. migratoria* suffers reduced growth and increased mortality due to their presence in food (Bernays & Chamberlain, 1980; Bernays et al., 1980). This background, together with the considerable amount known about acridid feeding biology (Bernays & Chapman, 1978; Simpson & Bernays, 1983; Bernays, 1985), made the comparison of *L. migratoria* and *S. gregaria* a convenient model system for the kind of study reported here.

Materials and Methods

Experimental Insects

Locusta migratoria and *Schistocerca gregaria* were reared under similar conditions (Hunter-Jones, 1961) at the Department of Zoology, Oxford University using seedling wheat and wheat bran as a food source. Insects were collected from the stock cages at the beginning of the fifth stadium (termed Day 0) so that their age fell within a 6-h range and their weights fell within 1 standard deviation of the mean of a large sample previously weighed. For *Locusta*

this weight range was 440-550 mg (males) and 520-650 mg (females), and for *Schistocerca* 529-634 mg and 602-737 mg for males and females respectively. The insects were then placed individually in clear plastic containers and provided with ample seedling wheat and wheat bran, which were replaced daily until the start of Day 3. The plastic containers used in two-choice experiments (28x16x9 cm) were larger than those used in no-choice experiments (17x12x6 cm), to accommodate a second Petri dish containing diet. The containers had in them a strip of expanded aluminium to provided a perch, and were kept in a constant environment room at 30°C under a LD 12:12 h photoregime. Even illumination was provided during light phases by overhead fluorescent strip lights.

Artificial diets

The nutrient composition of the four dry, granular, cellulose-based artificial diets used was as in Simpson & Abisgold (1985): PC - 28% protein, 28% digestible carbohydrate; Pc - 28% protein, 14% digestible carbohydrate; pC - 14% protein, 28% digestible carbohydrate, and pc which had 14% of both nutrients.

The commercial brand of tannic acid used (Sigma Ltd) may contain up to 8% impurities, including free glucose and gallic acid. However, feeding experiments using pure tannic acid (BDH Ltd) later confirmed that it is the tannic acid

and not the impurities which account for results reported here. The tannic acid powder (10% by dry weight) was added to the test diets only after they had dried to avoid complexing with protein and cellulose (Mole & Waterman, 1987) before being ingested by the insects. This meant that the tannic acid would form a surface coating over the lumps of diet, resulting in a variable ratio of tannic acid to diet depending on the size of individual lumps. The dry diet was therefore passed through wire sieves to produce particles of a standard size (425-850 um) before adding the tannic acid. At the 10% level, however, not all the tannic acid adhered to the lumps of diet, with some settling at the bottom of the feeding dishes during the course of the experiments. Weighing of the settled tannic acid suggested that the actual concentration in the diet was no less than 7.5%.

Locusts compensate for low protein levels in their food by eating more of diets containing 14% than 28% protein (Simpson & Abisgold, 1985; Abisgold & Simpson, 1987, 1988). It was therefore necessary to ensure that any observed effects resulting from the addition of tannic acid to the test diets were due to the chemical properties of tannic acid and not simply to the dilution of protein. Dilution of the control diet with additional cellulose was considered inappropriate because volumetric feedback from the gut plays an important role in determining meal size in locusts

(Bernays & Chapman, 1973; Simpson, 1983), and the bulky cellulose fibres would contribute a disproportionate effect compared to that due to the finely powdered tannic acid. The slight uncertainty regarding the exact concentration of tannic acid in the food ingested by the insects further justified this decision. To control for the dilution of protein due to the addition of tannic acid to the test diets, the data for amounts eaten over 12 h, and individual 24-h periods in the longer-term experiment on *Schistocerca* (Fig 3.), were therefore corrected for the maximum possible dilution effect (assuming 10% tannic acid and 100% compensation by the insects for the dilution of dietary protein). Whether or not this correction was made had no effect on statistical significance in any of the tests reported. Because of this, the same correction was not performed on the data for cumulative amounts eaten in the longer-term experiment on *Schistocerca* (Fig. 3). In such a case, the error introduced by the conservative nature of this step would compound with each successive addition of amounts eaten on subsequent days.

Amounts eaten

Shortly before the start of the light phase of Day 3, the insects were weighed and placed in clean plastic containers along with a 5.5 cm Petri dish (no-choice experiments) containing artificial diet (approximately 1.5 g). For two-

choice experiments, each Petri dish was glued to the centre of a larger Petri dish (9 cm), thus forming a central dish surrounded by a moat to prevent the spillage and mixing of the test and control diets. Before each experiment the dishes of diet had been dried in an oven at 45°C until they reached a constant weight (for 24 h) and weighed to within 1 mg, then placed open in the constant environment chamber for 12 h to equilibrate to ambient relative humidity; the equilibrated diets contained a maximum of 4% water. After the experiment the dishes were again dried and re-weighed to obtain a measure of the amounts eaten.

When the insects were weighed at the start of the experiments, they had an unknown amount of residual wheat and wheat bran in the digestive tract. This had cleared the gut by the end of the experiment and wheat and wheat bran faeces were easily distinguishable from artificial diet ones. To obtain an accurate measure of insect weight, the wheat and wheat bran faeces were therefore dried and weighed after the experiment, and the equivalent fresh weight (as obtained from a previously prepared regression equation of frass dry weight versus fresh weight) was subtracted from the weight of each insect.

Statistical analyses were performed using ANCOVA, with sex and tannic acid (absent or 10%) and, where appropriate, protein and carbohydrate (14% or 28%) as main effects and insect weight as a covariate. For two-choice experiments,

the term [amount eaten (control diet - tannic acid diet)] was calculated for each insect and the mean for all insects was tested using a one-sample t-test for significant difference from 0. For the longer-term experiment (see Fig. 3.), ANCOVA with diet as a main effect and insect weight at the start of the experiment as a covariate was used to test for differences in cumulative amounts eaten of test and control diets. To test amounts eaten over each individual period, the same design was used but with insect weight at the preceding measurement as a covariate.

Behavioural observations

Insects were observed for 12 h and the behaviour of individuals recorded once a minute. The cages were screened from each other using white cardboard strips. Three categories of behaviour were recognised: feeding (and in a two-choice situation, it was noted on which diet), locomotion and quiescence. In subsequent analysis of these data, intermeal intervals were distinguished from intrameal pauses by the use of bout criteria obtained using log-survivorship functions (Simpson, 1982). The pooled data for each of the four experimental groups (males and females feeding on control and test diets) were analyzed separately. The bout criteria ranged between 2 and 3 min., with a majority being 2 min. In this way it was possible to obtain the following measures of individual insect feeding

behaviour: Latency period before the first meal; duration of the first meal (from the start of feeding until the first pause of longer than 2 min. - i.e., including intrameal pauses (see Simpson, 1990)); number of meals; average meal duration; average intermeal interval; average meal size and mean ingestion rate (amount eaten/total time spent feeding).

These behavioural parameters, as well as total amounts eaten, were analyzed statistically using a 2x2 factorial ANOVA with sex and diet (0% or 10% tannic acid) as main effects. In addition, trends over time may be represented for use in the same ANOVA design as coefficients from the best fitting quadratic curve for the plot of parameter value versus time since the beginning of the first meal (Simpson & Abisgold, 1985). Best fit equations were obtained individually for each insect using the least squares method. For two-choice experiments, the same statistical test described above for total amounts eaten was used to test for significant differences in behavioural parameters.

Experiments

Interactions between tannic acid and nutrients.

To investigate possible interactive effects between protein, digestible carbohydrate and tannic acid on total amounts eaten during a 12-h period, 80 male and 80 female *Locusta* and 60 male and 60 female *Schistocerca* were given one of 8 diet mixtures: PC, Pc, pC, or pc each with or without 10%

tannic acid. For logistical reasons, the experiment on *Locusta* was conducted in 4 stages of 40 insects each, and *Schistocerca* in two stages, one of 40 and one of 80 insects. Equal numbers of each sex were used in each stage, and treatments were as far as possible distributed equally between the stages. Using ANCOVA it was shown that 'experimental replicate' (with four levels for *Locusta*, and two for *Schistocerca*) made no significant contribution, either as a main effect or in two-way interaction terms, towards explaining the variance in the dependent variable, amount eaten. Males and females were positioned alternately, and equal numbers of each diet were allocated randomly within each sex.

Behavioural observations, no-choice.

In order to investigate in detail the effect of tannic acid on the feeding behaviour of the insects, 32 insects (16 males and 16 females) of both species were offered pc diet with or without 10% tannic acid. The species were tested separately in groups of 32 insects, and their detailed behaviour was recorded and total amounts eaten measured over a 12-h period. Males and females were positioned alternately, and the control and test diets were allocated alternately within each sex.

Two-choice experiments.

In this series of experiments, 22 insects (11 males and 11 females) of each species were offered a choice of pc diet with and without 10% tannic acid, and the total amounts of each diet eaten over a 12-h period were measured. This experiment was performed a second time for *Locusta*, while recording patterns of feeding behaviour.

Longer-term experiment.

To investigate whether the effects of tannic acid on feeding persisted beyond 12 h, 20 *Schistocerca* females were given pc diet with or without 10% tannic acid at the start of Day 3 and their progress was monitored through the remainder of the fifth stadium. Amounts eaten were measured and fresh diet was provided at the end of that light phase (12 h), then again at the end of the following dark phase, and at 24-h intervals thereafter until all the insects had moulted. The insects were weighed at 24-h intervals, but no account could be taken of residual food in the digestive tract. However, an accurate measure of total growth through the experimental period was obtained by weighing the insects while their guts were still empty after they had moulted to the adult stage. The duration of the stadium for each insect was recorded to the nearest 12 h.

In the short-term (12 h) experiments, insects were not provided with free water (see Simpson & Abisgold, 1985).

However, for periods of longer than this water is required in addition to the dry diets, and this was provided in rectangular plastic containers (7.8x4.7x2.0 cm) which had two 1.5 cm holes drilled into their lids. As a control to verify that the absence of water really was not important in short-term experiments, insects were kept without as well as with water for 48 h. The absence of water caused no difference in amounts of pc diet (with and without tannic acid) eaten over this period.

Results

Interactions between tannic acid and nutrients

Table 1 shows the results of the experiment in which protein, carbohydrate and tannic acid levels were varied simultaneously. Tannic acid as a main effect had no measurable influence on total amount eaten by *Locusta*, but caused a significant increase in total consumption by *Schistocerca* (Fig 1). Significantly more of the low protein (p) than the high protein (P) diets were eaten by both species, thus confirming the results of Simpson & Abisgold (1985) for *L. migratoria*, and demonstrating that *S. gregaria*, too, regulates food intake to compensate for dietary protein levels. Total amounts eaten (mean$\pm$SE) by *Locusta* were 78.6$\pm$2.6 mg of p-diets and 62.2$\pm$2.4 mg of the P-diets, while *Schistocerca* ate 110.5$\pm$5.3 mg and 84.5$\pm$3.9

TABLE 1. Summary of F-ratios from the ANCOVA for amounts eaten by fifth stadium locust nymphs of diets with two levels of protein, digestible carbohydrate (14% or 28%) and tannic acid (absent or 10% by dry weight). *P<0.05; ***P<0.001.

Source	F-values	
	Locusta	*Schistocerca*
Covariate		
Insect weight	4.1*	4.6*
Main effects		
Sex (S)	0.2	0.1
Tannic acid (T)	1.6	42.8***
Protein (P)	31.2***	20.3***
Carbohydrate (C)	5.4*	0.1
Two-way interactions		
S x T	1.1	<0.1
S x P	0.2	0.8
S x C	4.0*	0.2
T x P	1.3	<0.1
T x C	1.3	<0.1
P x C	1.3	1.8

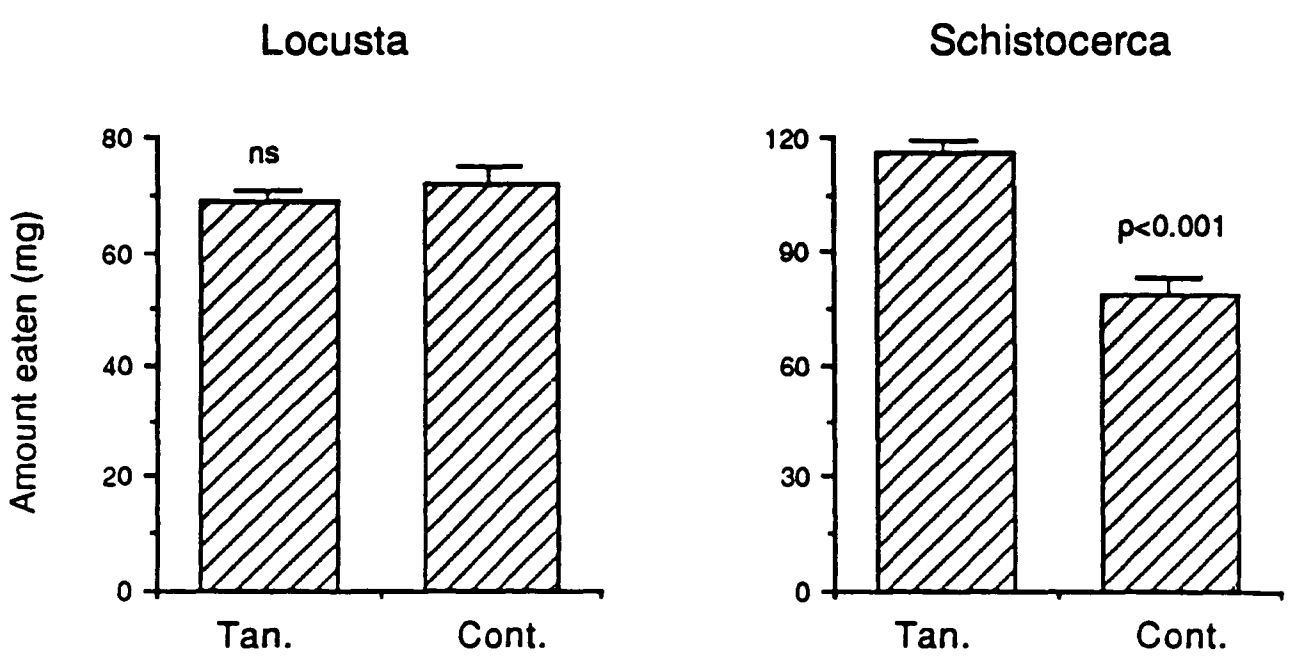


FIG. 1. Total amounts eaten (mean±SE) over 12 h by fifth stadium locust nymphs of PC, Pc, pC and pc diets (see text) with (tan.) or without (cont.) 10% tannic acid.

mg of the p and P-diets respectively. There were no main effects of dietary carbohydrate for *Schistocerca* or female *Locusta* but, contrary to the findings of Simpson & Abisgold (1985), there were for *Locusta* males. The males ate more of the low (69±2.9 mg) than the high (55±3.2 mg) carbohydrate diets; the equivalent figures for females were 78.9±5.3 mg and 78.4±3.3 mg, respectively. The fact that this effect due to carbohydrate is only seen in one case (in male *Locusta* in the present study) suggests that, over a 12-h period at least, it is of marginal importance relative to compensatory feeding for protein. There were no significant interaction terms for either species between tannic acid and protein or carbohydrate levels or between tannic acid and sex. Clearly, then, increased consumption of tannic acid due to compensation for low dietary nutrients did not result in a difference in the effect of the allelochemical.

Behavioural observations, no choice

The presence of tannic acid in pc diet caused a significant increase in total amounts eaten over 12 h by *Schistocerca* (Table 2, Fig. 2a), confirming the results obtained in the previous experiment. This increase was primarily due to the insects taking significantly more meals (Fig. 2b), by shortening the average intermeal interval (Fig. 2c). There was no significant difference in the size of meals taken on the two diets (Fig. 2d). The presence of tannic acid also

TABLE 2. Summary of F-ratios from the ANCOVA for various behavioural parameters for fifth stadium locusts given pc diet (see text) with or without 10% tannic acid. *P<0.05; **P<0.01; ***P<0.001

Source	F-values							
	Amount eaten	Number of meals	Average intermeal interval	Average meal size	Average meal duration	Ingestion rate (amount eaten/time feeding)	Time to first meal	Duration of first meal
Locusta								
Covariate								
Insect weight	0.3	0.6	1.7	1.8	6.1*	11.0**	2.2	0.2
Main effects								
Sex	0.3	0.4	1.6	3.9	7.8**	8.1**	1.0	1.5
Tannic acid	0.7	1.2	0.6	1.2	3.0	2.1	0.1	0.8
Two-way interaction								
Sex x Tan	0.6	0.1	<0.1	<0.1	<0.1	0.1	<0.1	0.3
Schistocerca								
Covariate								
Insect weight	0.4	0.1	<0.1	<0.1	<0.1	<0.1	0.3	<0.1
Main effects								
Sex	0.1	0.8	0.1	1.3	1.1	2.2	0.3	0.1
Tannic acid	12.6**	22.8***	7.1*	2.5	2.8	<0.1	5.9*	4.6*
Two-way interaction								
Sex x Tan	0.8	1.1	0.6	2.0	1.5	2.4	1.4	3.9

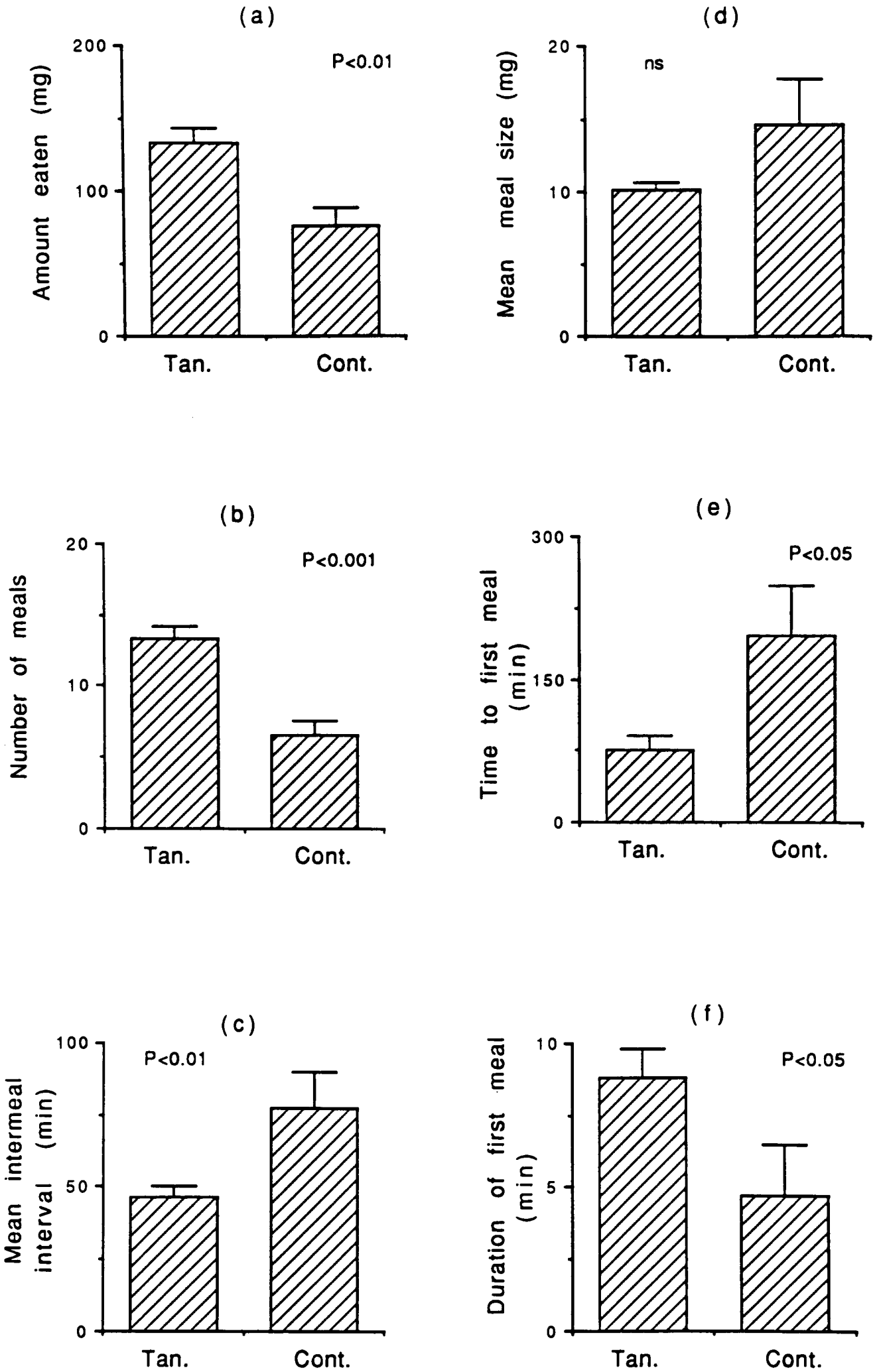


FIG. 2. Values of various behavioural parameters (mean±SE) for fifth stadium *Schistocerca* nymphs fed pc diet (see text) with (tan.) or without (cont.) 10% tannic acid.

caused a significant reduction in the latency period before the first meal (Fig. 2e) and an increase in the duration of the first meal (Fig. 2f). Since the insects at this stage had never before contacted either artificial diet, this suggests that tannic acid caused increased consumption through a phagostimulatory effect, rather than through some post-ingestive feedback mechanism.

Locusta showed no significant change due to tannic acid in any of the behavioural variables measured (Table 2). There was, however, a significant sex effect on average meal duration, with females taking on average longer meals than males (5.1 and 2.4 min., respectively). Longer meals did not result in increased meal size however, because the average consumption rate of males (2.0 mg/min) was significantly higher than that of females (1.1 mg/min; these figures, as well as those for average meal duration above, represent means adjusted for the significant effect of the covariate insect weight).

There was no significant effect due to tannic acid on the quadratic or linear coefficients for meal duration or intermeal interval versus time since the first meal, indicating that the trends over time with respect to these parameters were the same on both diets (Simpson & Abisgold, 1985).

Two-choice experiments

When given a choice between control and tannic acid diets, *Schistocerca* ate more of the tannic acid diet (Table 3), confirming that this compound causes increased consumption through direct peripheral sensory stimulation rather than through post-ingestive feedback. *Locusta*, on the other hand, ate significantly more of the non-tannic acid diet in both the initial two-choice experiment and the repeat in which behavioural data were recorded (Table 3, experiments 1 and 2, respectively). The behavioural data in Table 3 show that this increased consumption was due to the insects taking meals more frequently on the control diet, which were of similar average size to those taken on the tannic acid diet. Significantly more of the insects took their first meal on the control diet ($P<0.001$; probability calculated using the binomial distribution), indicating a pre-ingestive deterrent effect due to tannic acid.

Longer-term experiment

Figure 3 shows the cumulative amounts eaten of control and tannic acid diets by *Schistocerca* females from the third day of the fifth stadium; the stadium duration of both groups is also represented. Over the first 12-h period, significantly more ($P<0.01$) of the tannic acid diet was eaten, as could be expected from results of the previous experiments. This effect persisted over the second 12-h

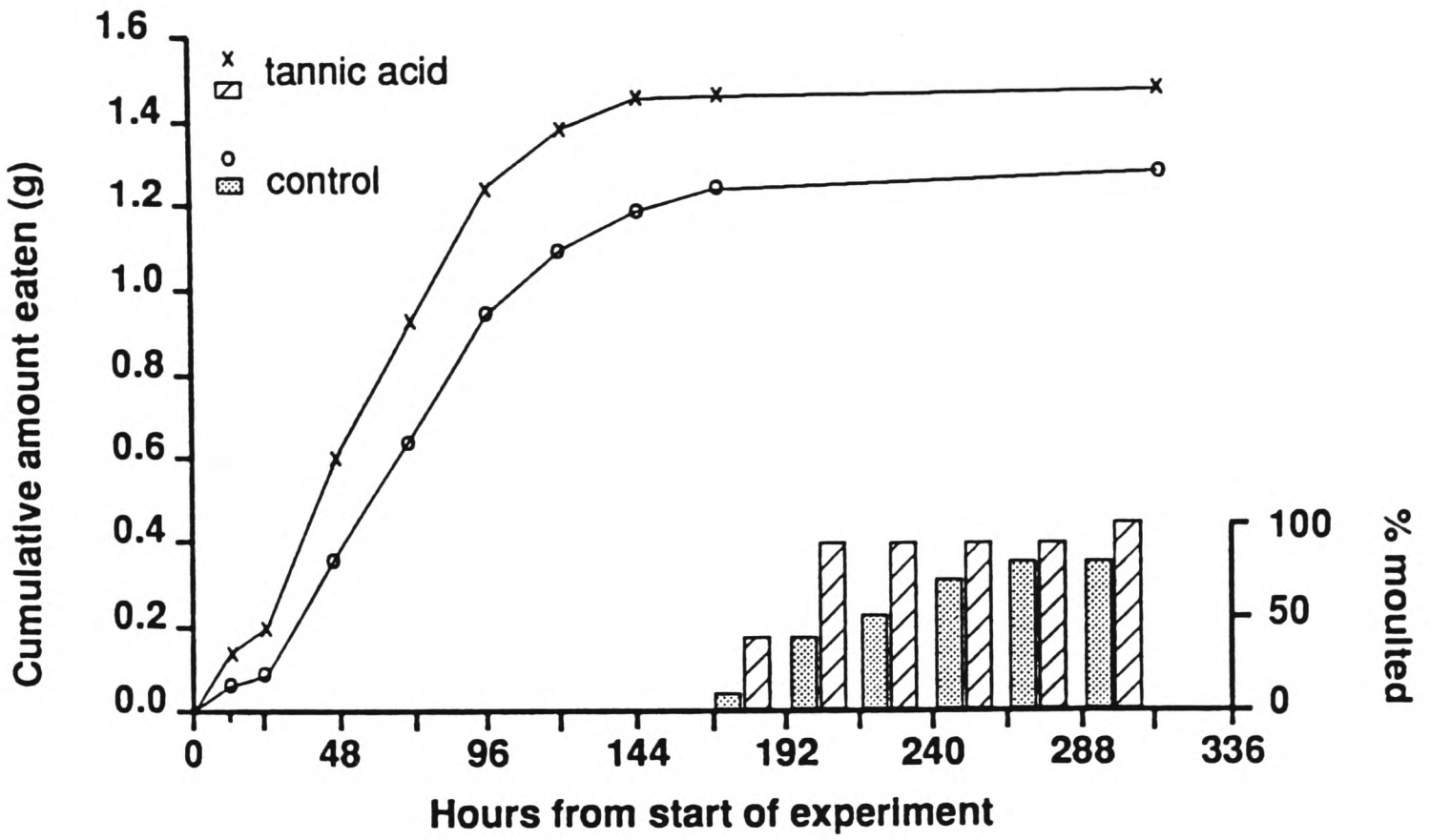


FIG. 3. Cumulative amounts eaten and the percentage moulted of fifth stadium *Schistocerca* nymphs fed pc diet (see text) with or without 10% tannic acid. Differences in cumulative amounts eaten of the test and control diets are significant at all points (using ANCOVA with two levels for diet and insect weight as a covariate). Amounts eaten of the two diets over individual periods differed significantly only for periods 0-12 h and 12-24 h (see text for levels of significance).

TABLE 3. Means (±SE) of the choice term (control diet - tannic acid diet) of various behavioural parameters of fifth stadium locusts given pc diet (see text) with and without 10% tannic acid. Results from two experiments with *Locusta* and one with *Schistocerca* are presented. P-values are for a two-tailed t-test of significance between means and 0, the expected value if there was no discrimination between the diets.

Behavioural parameter	[(Control diet) - (tannic acid diet)]	t	Sign.
Schistocerca			
Amount eaten (mg)	-51.6±11.1	4.6	P<0.001
Locusta			
Amount eaten (mg) (expt 1)	50.4±6.4	7.9	P<0.001
Amount eaten (mg) (expt 2)	34.5±8.4	4.1	P<0.001
Number of meals (expt 2)	7.8±1.6	5.0	P<0.001
Average meal size (expt 2)	-0.6±1.3	0.4	ns

period (the first dark phase in the experiment) ($P<0.01$), but thereafter there were no significant differences in amounts eaten over individual 24-h periods. However, the cumulative amounts eaten by the tannic acid-fed insects remained significantly greater throughout the rest of the experiment, indicating that the difference in amounts eaten due to enhanced consumption over the first 24 h of exposure to tannic acid diet were not made up by the control insects over the remainder of the fifth stadium. The probability levels for the successive measurements were as follows: 48 h, 72 h, 96 h, and 120 h ($P<0.01$); 144 h, 168 h, and the total amounts eaten after all the insects had moulted (hour 312) ($P<0.05$).

Total growth over the course of the experiment (from the beginning of Day 3) and relative growth rate were both significantly greater on the tannic acid diet ($P<0.05$; Fig. 4a and b). When the data for total growth were analyzed in an ANCOVA using total amount eaten as a covariate, the covariate was significant ($P<0.001$) and the effect of diet on total growth fell away ($P=0.3$). This suggests that the effect on growth of tannic acid was due to increased amounts eaten rather than to increased nutritional value of the food. There were also indications of shortened stadium duration due to tannic acid diet (Fig. 3), but this was not statistically significant. A control insect which had not moulted by 14 days after the rest was considered abnormal and excluded from the analysis.

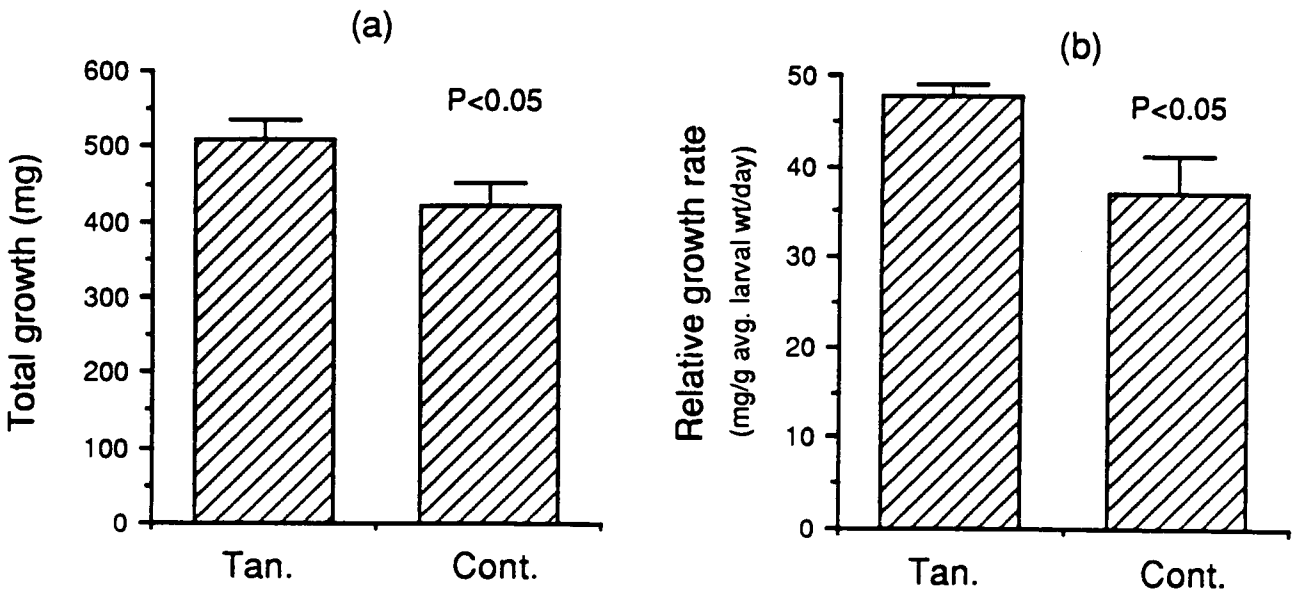


FIG. 4. Means (±SE) of total growth (a) and relative growth rate (total growth per day/average larval weight) (b) from the beginning of the third day of the fifth stadium to the final moult of female *Schistocerca* nymphs fed pc diet (see text) with (Tan.) or without (Cont.) 10% tannic acid.

Discussion

The concentrations of protein and tannic acid in the synthetic diets used in this study were chosen as realistic levels likely to be encountered in plants, and the stage during the stadium at which the insects were tested is a period of high feeding activity (Simpson, 1982). Digestible carbohydrate levels in the diets were rather higher than in real plants. While insects of both species increased consumption to compensate for low levels of protein, and male *Locusta* for carbohydrate, there were no significant interaction terms between tannic acid and nutrients. This indicates that, over a 12-h period, the presence of tannic acid in the diet does not affect the ability of *Locusta* or *Schistocerca* nymphs to compensate for reduced levels of nutrients by increasing consumption.

From a functional viewpoint, these results are not altogether surprising. The nutrient requirements of herbivorous insects and the quality and quantity of available food are continuously changing and compensatory feeding is an important mechanism whereby the insects maintain a nutritionally balanced diet (Simpson & Simpson, 1990). The ability to adjust food intake according to nutrient levels despite the presence in the food of plant allelochemicals would therefore be advantageous. However, depending on the toxicity of the allelochemical, a point may be reached at which the nutritional advantage of increased

intake of the food is offset by the deleterious effects of the allelochemical. In an insect adapted to cope with this relationship between nutrients and toxic chemicals in plants, it is at this point that compensatory feeding should be affected. It is known that in the long-term tannic acid has toxic effects on *L. migratoria* (Bernays, 1978), and in Chapter 2 I therefore address the effects on compensatory feeding by this species of prolonged exposure to tannic acid.

Previous studies into the effects on herbivorous insects of combined changes in dietary nutrients and allelochemicals have produced varied results. Hare (1987) raised larvae of the potato beetle (*Leptinotarsa decemlineata*) on artificial diets containing measured quantities of protein and one of five glycoalkaloids. For four of the alkaloids there was no significant interaction term with protein in larval weight gain, but for the most toxic, tomatine, there was. Johnson & Bentley (1988) examined the effects of protein and the alkaloid sparteine on growth and survivorship of larval *Spodoptera eridania*. The effects of protein and sparteine were independent when the artificial diet contained high levels of wheat germ, but when the major protein component was casein there was a significant interaction term. There was also an apparent interaction between protein and sparteine in influencing developmental rate, since the effects of protein concentration on larval stages beyond the

first stadium was primarily evident when sparteine was present at high concentrations. Unfortunately, neither Hare (1987) nor Johnson & Bentley (1988) measured consumption rates or other behavioural parameters of the larval stages for which a significant interaction term existed. It is therefore not possible to determine whether reduced performance was due to the toxic effects of the allelochemicals, or to reduced consumption as an adaptive behavioural response to maximise nutrient intake while minimising the toxic consequences of ingesting the allelochemicals.

Lincoln et al. (1982) studied the response of larval checkerspot butterflies (*Euphydryas chalcedona*) to varying concentrations of the leaf resin from their foodplant and of protein. While larval survivorship, growth rates and the size of larvae at diapause were enhanced with increasing protein and depressed with increasing resin concentrations, there were no significant multiplicative interactions between these factors. The larvae ate more of the low protein diets, indicating an attempt at dietary compensation. There were no direct effects of the resin on consumption rates, but total consumption was reduced through it effects on larval size, growth rate and survivorship. It is, however, difficult to determine the extent to which these data represent the situation in the field, since in the preparation of the artificial diets the dry ingredients

were mixed with water thus providing a medium in which the phenolic resins and proteins could form chemical complexes before the insects encountered the diet. The resins occur as a surface layer on the intact leaves of the host plant, so they would not normally contact soluble leaf proteins until after the insect bites the leaf. The experimental situation may therefore have had an important effect on the relationship between sensory input from resin and protein, and hence on the insects' behavioural response to these chemicals.

Slansky & Wheeler (unpublished data) found a significant interaction between caffeine and the degree of dietary dilution in affecting growth and survivorship of the velvetbean caterpillar (*Anticarsia gemmatalis*). Increased consumption to compensate for the lowered nutrient levels in the diluted diets resulted in the ingestion by the insects of deleterious quantities of caffeine. There was no significant effect on relative consumption rate, indicating that reduced total consumption was a secondary effect of reduced growth rather than due to a deterrent effect of caffeine on feeding.

In the present study, in addition to a lack of interactions between tannic acid and nutrients, there were no interactive effects of tannic acid with sex. However, male and female *Locusta* responded differently to dietary carbohydrate levels, with only the males showing a weak

compensatory response to reduced dietary carbohydrates. Also, the patterns of feeding differed between the sexes, with the males of this species taking on average shorter meals at a higher ingestion rate, but this was independent of the presence of tannic acid. Despite the lack of interactions of tannic acid with dietary nutrients or sex, it is none the less clear that tannic acid did in itself have a profound, but different, effect on the feeding behaviour of *L. migratoria* and *S. gregaria*.

Locusta

In a no-choice situation there were no measurable effects of tannic acid on the feeding behaviour of *Locusta*, but when given a choice the insects took fewer meals thereby decreasing consumption on the tannic acid diet relative to the control. These results do not accord with those of Bernays & Chapman (1977), who found that in a no-choice situation 1.2% tannic acid applied to cornflour wafers caused a 50% reduction in amount eaten over a 1 to 2-h period by fifth stadium *Locusta* nymphs. However, cornflour wafers are considerably less phagostimulatory than the diets used in the present study, and this probably explains why the insects were deterred by tannic acid in that study and not in the no-choice experiments in the present study. In the present two-choice experiments, significantly more of the naive insects took their first meal on the control diet,

indicating a pre-ingestive deterrent effect of tannic acid. Such deterrence could be due to responses from specific deterrent receptors, or to interference with receptors responsible for phagostimulation. For example, tannic acid is known to deter feeding in some lepidopterous larvae by inhibiting the sugar receptors responsible for phagostimulation (Dethier, 1982; see also Mitchell & Sutcliffe, 1984). Whatever the sensory mechanism, it is known that there are physiological costs associated with the ingestion of tannic acid for *L. migratoria* (Bernays, 1978; Bernays et al. 1980), and it would therefore be adaptive if, when a choice is available, these insects avoid consuming food containing this compound.

In accordance with the results of the present study, Bernays & Chamberlain (1982) found that in a choice assay *L. migratoria* nymphs showed a preference for controls over wheat leaves which had a surface coating of tannic acid (10% and 20%), and this effect was not apparent in a no-choice test. It is not surprising that such an effect may be found only in a choice test, since this form of assay is known to be more sensitive than are no-choice tests in which only total consumption is measured (Cook, 1976). However, that the effect was not seen in the patterns of feeding in the no-choice assay in the present study is more surprising. For instance, Barton Browne et al. (1991) found that analysis of feeding patterns in a no-choice test was a more sensitive

indicator of deterrence for the Australian sheep blowfly than choice and no-choice assay in which only total amounts eaten were measured.

A partial explanation for the lack of such an effect in the present study is suggested by considering the mechanisms whereby a feeding deterrent can possibly influence the patterns of feeding. Initiation and termination of feeding in locusts are determined, all else being equal, by the balance between the stimulatory properties of the food and the inhibition from gut stretch receptors and blood composition due to the previous meal (Simpson, 1990). A feeding deterrent can raise the threshold for initiating feeding, so that feeding begins only when the inhibitory feedback from the gut and blood is lower, or it can lower the threshold for meal termination, so that the insect stops feeding at a lower gut volume. Both possibilities may on their own result in reduced consumption; in the first case this would be due to the insect taking meals less frequently than it would in the absence of the deterrent, and in the second case, to a reduction in the size of the meals taken. However, if the thresholds for initiation and termination of feeding are altered equally, such that the mean difference between the gut volume at which feeding begins and ends is left unchanged, then the patterns of feeding would be expected to be unaffected by the feeding deterrent (Barton Browne et al., 1991). This possibly explains why,

in the no-choice assay, there were no differences between tannic acid and control insects in the amounts eaten, number of meals, meal size and intermeal intervals, but it does not explain why there was no difference in the latency period before the first meal. A raised threshold for the initiation of feeding due to tannic acid should result in the test insects starting to feed later than the controls; or, as the results for the choice experiments illustrated, in the first meal being taken on the less inhibitory of two diets.

My data are insufficient to explain this interesting phenomenon, but it seems that any differences in latency to feed in no-choice tests were hidden by the variability between insects and the fact that latencies on the diets were long relative to insects fed wheat (Simpson, 1982). Both tannic acid and control diets were initially novel to the insects and, apparently, to some extent deterrent.

Irrespective of the mechanism, it is interesting that a chemical which was shown in the choice experiments to be a feeding deterrent did not result in a reduction in the overall amount eaten when no choice was available. Such behaviour might be adaptive since it would enable the insects to exercise strong discretion where a choice of foodplants is available, yet make the most of a sub-optimal food source otherwise. These results illustrate how subtle the effects of feeding deterrents can be, and emphasise the value of detailed behavioural observations as well as the

need to perform both choice and no-choice tests in studies of this kind (see also Schoonhoven, 1982).

Schistocerca

Schistocerca ate more of the tannic acid than the control diets in both the choice and no-choice experiments, this increased consumption being due to a shortening of the intermeal interval. That the naive tannic acid-fed insects started feeding sooner and the first meal was of a longer duration than in the controls, indicates a pre-ingestive stimulatory effect due to tannic acid. This does not accord with the results of Bernays & Chamberlain (1980). They found that for *S. gregaria* nymphs, consumption of artificial diet was reduced by the presence of 18% tannic acid over the first day of the fifth stadium. However, in the preparation of the diet the mixture was wet after combining the tannic acid with 18% protein, thus providing a medium in which the tannic acid and protein could react forming insoluble complexes at the favourable ratio of 1:1 (Goldstein & Swain, 1965). Sixty percent of the protein was casein, which is known to react with tannins (Gstirner & Korf, 1966; Feeny, 1968). Therefore, the tannic acid would not have been available to the chemoreceptors to cause increased consumption, but it is uncertain how complexed tannic acid and protein could actually reduce consumption. While proteins are themselves not known to be stimulatory, some

amino acids are (Cook, 1977) and precipitation of these with the tannin (Mole & Waterman, 1987) might have reduced the stimulatory properties of the test diet. Furthermore, it is known that diets lacking protein altogether are eaten in small amounts (Simpson et al. 1988), and it could therefore be that consumption was reduced because complexing effectively removed the protein from the diet. It is perhaps significant in this regard that no reduction in consumption occurred when Bernays & Chamberlain (1980) applied the tannic acid (20%) to the surface of wheat where it could not contact the soluble leaf proteins. Also, since in that study there was no reduction in consumption of wheat containing 20% tannic acid, it seems unlikely that the difference between the results of the artificial diet studies of Bernays & Chamberlain (1980) and those of the present study is due to the different concentrations of tannic acid used in the two studies (18% and 10%, respectively). On the other hand Bernays & Chamberlain (1980) found no reduction in growth due to the presence of tannic acid in the artificial diet, and presumably therefore in available protein, and this might at first sight appear not to support the possibility that reduced consumption was due to the formation of tannin-protein complexes. However, tannin-protein complexes are known to be reversible by surfactants (Goldstein & Swain, 1965) such as those found in the gut of *S. gregaria* (Martin et al., 1987), so that any complexed

protein in the diet would none the less be made available to the digestive enzymes. Indeed, there is evidence that tannins can, under certain conditions, induce conformational changes in the structure of proteins increasing their digestibility by tryptic enzymes (Mole & Waterman, 1985).

Bernays & Chamberlain (1980) found that the reduced consumption due to tannic acid in artificial diet persisted for only 24 h, after which consumption was similar to that on the control diet. Similarly, in the present work, the stimulatory effects of tannic acid on *Schistocerca* nymphs also persisted for only 24 h. This indicates that behavioural habituation may occur not only to feeding deterrents (Szentesi & Bernays, 1984), but also to non-nutrient feeding stimulants.

Despite the waning after 24 h of the stimulatory effects of tannic acid in the present study, the insects fed diet containing this compound achieved a significantly greater adult weight and a slightly shorter stadium duration than the controls. Similarly, Bernays (1978) and Bernays & Chamberlain (1980) found that *S. gregaria* nymphs fed wheat containing a surface coating of 20% tannic acid grew significantly more than controls over the fifth stadium with indications of reduced stadium duration. Both consumption and the efficiency of conversion of digested food were slightly higher for the tannic acid-fed insects in that study, but neither was significantly so. Bernays (1978)

found that approximately two thirds of the tannic acid was hydrolysed on its passage through the gut and suggested that *S. gregaria* might benefit from the sugars released during this hydrolysis. The present work has demonstrated that increased consumption which occurs during the first 24 h of exposure to tannic acid can account for the increased growth over the fifth stadium.

On the other hand, Bernays (1978) found no significant increase due to tannic acid in total growth through all the larval stadia of *S. gregaria*. From this it appears that the transitory effect on consumption may be beneficial to growth over a limited period only, at least when the insects are exposed to tannic acid continuously. It will be interesting to see whether the stimulatory effect can be reset by alternately feeding the insects tannic acid and control diets. This would have important implications for foraging theory, since it might provide a selective basis other than dietary self-selection (Waldbauer & Friedman, 1988) for the frequent switching of plants while feeding by polyphagous insects.

While it is known that tannic acid stimulates feeding in the larvae of some tree feeding Lepidoptera (Bernays, 1981), the same has not hitherto been demonstrated for orthopterans. At least one species of acridid, *Anacridium melanorhodon*, is known to benefit from ingesting this compound (Bernays et al. 1980; Bernays & Woodhead, 1982),

but in the present study there were no indications of a nutritional effect on *S. gregaria* over and above the increased consumption. This is surprising since compounds which stimulate feeding by insects are often themselves nutritious or otherwise beneficial to the insect, or signal the presence in plants of such compounds. Perhaps insects stimulated to increase consumption of diets containing tannins have evolved to do so to compensate for some costs associated with the detoxification of these compounds. For example, acridids that regularly ingest tannins have more substantial peritrophic membranes which can, in extreme cases, entail an investment of up to 10% of ingested protein (Bernays & Simpson, 1990). D. Raubenheimer and S.J. Simpson (unpublished) found that, when deprived of water, *S. gregaria* nymphs fed pc diet containing 10% tannic acid lost significantly more weight over a 48-h period than controls, despite the fact that their cumulative consumption remained higher than that of the controls. This suggests that there may well be physiological costs due to ingesting tannic acid, at least when water is absent. Additionally or alternatively, there may be ecological advantages to the ingestion of tannins, and these would not be apparent in laboratory studies of the kind reported here.

In conclusion, detailed observations of the insects' behavioural response to manipulation of chemicals in artificial diets has provided new insights into the effects

of tannic acid on feeding by *S. gregaria* and *L. migratoria*, and has identified several unanswered questions regarding the mechanisms whereby insects respond to allelochemicals. Some of these will be further addressed in Chapter 2.

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Chapter 2

Tannic acid, protein and digestible carbohydrate: dietary imbalance and nutritional compensation in the African migratory locust

Under revision: *Ecology*.

Abstract. The combined effects of dietary imbalance and the allelochemical tannic acid on fifth stadium *Locusta migratoria* were investigated. In a factorial-design experiment, insects were fed artificial diets containing digestible carbohydrates and proteins in equal proportions (14% or 28%), or in a 1:2 or 2:1 ratio, with or without 10% tannic acid. Growth, consumption and utilization efficiencies were measured over the course of the fifth stadium and histological analyses of the midguts of newly molted adults were undertaken.

While the consumption of diet was higher on the 14%-protein diets, consumption of protein remained lower than on the 28%-protein diets indicating that compensatory feeding for protein was incomplete. Similarly, compensation for low dietary carbohydrates occurred, but was incomplete. Low levels of either nutrient resulted in increased intake of the other, but the effect of protein on carbohydrate intake was stronger than vice-versa. There was an interactive effect of carbohydrate and protein on the amounts of carbohydrate consumed, resulting from the fact that particularly high levels of carbohydrate were consumed when the diets simultaneously contained 28% carbohydrate and 14% protein. The terms 'incidental augmentation' and 'incidental restriction' of intake are introduced to describe the ways that certain nutrient groups may affect the intake of others in diets which are imbalanced.

Tannic acid resulted in reduced efficiency of conversion of ingested nitrogen, but the efficiency of conversion of total ingested food was higher on the tannic acid diets. A higher frequency of lesions was observed in the midgut epithelia of tannin-fed insects than controls, and it is suggested that the sloughing of necrotic epithelial tissues into the lumen and their subsequent egestion with the faeces may account for the reduced efficiency of nitrogen conversion in these insects. A statistical interaction between protein levels and tannic acid demonstrated that tannic acid reduced consumption, but only of the low protein diets. Therefore, the effect of tannic acid was to restrict compensatory feeding for protein. A consequence of this was reduced growth in insects fed low-protein diets containing tannic acid.

Total dry weight growth was lower on the 28%-protein diets and higher on the 28%-carbohydrate diets, and therefore corresponded with levels of carbohydrate intake. Nitrogen accumulation increased with dietary protein levels, but remained constant across carbohydrate levels despite increased intake of nitrogen on low-carbohydrate diets. This was accounted for by lower efficiency of conversion of nitrogen on the 14%-carbohydrate diets. Approximate digestibility was higher for the 14%-protein diets, possibly reflecting increased intake of easily digested carbohydrates. There were no statistically significant

interactive effects between nutrients or nutrients and tannic acid on utilization efficiencies.

INTRODUCTION

The availability and balance of nutrients in plant tissue may vary widely in relation to the dietary requirements of herbivorous insects (House 1969). An insect encountering a potential foodplant containing low levels of, for example, nitrogenous nutrients may reject the plant or compensate for its poor nutritional quality by altering the efficiency with which it acquires or processes the limiting nutrient. Such compensation may be behavioral, as in increased consumption, physiological (increased digestion, absorption or conversion) or some combination of these (Slansky and Scriber 1985, Simpson and Simpson 1990). Compensation is therefore an important means whereby insects maintain nutritional homeostasis in the face of dietary imbalance or food shortages.

However, an insect cannot regulate the intake of one compound without simultaneously altering intake of all others. It may, therefore, be an over-simplification to consider in isolation the role of individual compounds in regulating dietary intake by insects. For example, increased consumption by an insect to compensate for low levels of protein in a nutritionally imbalanced food may be restricted

by the insect's ability to cope with the simultaneous increase in the levels of other nutrients ingested. Likewise, compensatory feeding may lead to the increased ingestion of toxic allelochemicals (Sen Gupta and Miles 1975, Hare 1987, Johnson and Bentley 1988). A corollary of the latter point is that the effectiveness of an allelochemical as a defence against herbivory may be dependent on the levels of nutrients present in the plant tissue.

There are, additionally, several examples of interactions between coingested compounds in imbalanced diets affecting nutritional physiology. Horie and Watanabe (1983) found, by supplementing low-quality protein diets with free amino acids, that the utilization of certain amino acids by silkworm larvae was influenced by the levels of others present in the hemolymph. Bloem et al. (1989) found that *Heliothis zea* larvae experienced reduced conversion of digested food (ECD) when the glycoalkaloid tomatine was present, but only when the diet simultaneously lacked cholesterol. Broadway and Duffey (1988) demonstrated that the quality of dietary proteins ingested by *Spodoptera eridania* larvae altered the toxicity of soybean trypsin inhibitor in artificial diets.

Consideration of such interactive effects may be particularly important in cases where correlations are found to exist in nature between the levels of particular

compounds in plant tissues. For example, several studies have found an inverse relationship between the levels of nitrogen and phenolic compounds in leaves (e.g. Kiraly 1976, Phillips and Henshaw 1979, Mattson 1980, Miles et al. 1982, Tuomi et al. 1984, Ohmart et al. 1985, Price et al. 1989). The carbon-nutrient balance hypothesis has been proposed to explain this from the proximal viewpoint of the plants (see Discussion), and it has been suggested that a low ratio of nitrogen to carbon-based allelochemicals may have evolved as plant defenses in low-nutrient habitats (Feeny 1975, Bryant et al. 1983). There has, however, been little attention paid to how an inverse correlation between dietary nitrogen and phenolics may affect the feeding behavior and physiology of individual herbivorous insects.

I therefore initiated a recent study into the interactive effects on the African migratory locust (*Locusta migratoria*) of simultaneous changes in the levels of protein, carbohydrates and tannic acid in artificial diets (Chapter 1). It was found that, over a 12-h period, the ability of fifth instar nymphs to compensate for low levels of dietary proteins and carbohydrates by increasing consumption was unaffected by the presence in the food of 10% tannic acid. There was no main effect due to tannic acid and no apparent interactive effects between the nutrients or between tannic acid and either of the nutrients.

The present study was designed to examine, over the

longer term (throughout the fifth stadium), the combined effects of tannic acid and nutritional imbalance on aspects of the feeding behavior and physiology of *L. migratoria*. Protein and carbohydrates were paired in chemically defined diets either in balanced proportions (14% or 28% of both) or in skewed proportions (14% of one and 28% of the other) with or without 10% tannic acid, and the effects on consumption, growth and utilization efficiencies were measured.

METHODS

Insects and diets

Experimental insects were reared at the Department of Zoology, Oxford University using seedling wheat and wheat bran as a food source. Insects were collected from the stock cages at the beginning of the fifth stadium so that their age fell within a 6-h range and their weights fell within 1 standard deviation of the mean of a large sample previously weighed (440-550 mg for males and 520-650 mg for females).

The eight dry, granular, cellulose-based artificial diets used were as in Chapter 1: PC, Pc, pC and pc (where "P" and "p" represent 28% and 14% protein, and "C" and "c" 28% and 14% digestible carbohydrate, respectively), each with or without 10% pure tannic acid (BDH Ltd.). Dadd (1960a) found

that 13% carbohydrates combined with 27% proteins in artificial diets did not support optimal growth and development of *Locusta migratoria*, and based on this the PC and pc diets were considered in the design of the present experiment to be more balanced than the Pc or pC diets. This assumption is further supported by the fact that the ingestion of nutrients was regulated around a similar point for diets with equal levels of protein and carbohydrates, but diverged considerably for diets containing these nutrients in skewed proportions (see Fig. 1).

Experimental

Twenty four newly molted insects of each sex were selected according to the weight and age criteria above, placed together in a single 10 l container with an excess of seedling wheat and bran and left overnight in a constant environment experimental room (30°C, under a L:D 12:12 h photoregime). Shortly before the start of the following light phase (i.e., the light phase of Day 1), each insect was weighed and placed in a separate clear plastic box (17x12x6 cm) containing an expanded aluminum perch and, to provide water, a rectangular plastic container (7.8x4.7x2.0 cm) with two 1.5 cm holes drilled in its lid. Males and females were positioned alternately.

To estimate insect fresh weight at the start of Day 1, a correction had to be made for wheat and bran faeces

residual in the gut. These (easily distinguishable from artificial diet faeces) were therefore collected from the boxes at the start of Day 2, dried, weighed and the equivalent fresh weight (as obtained from a previously constructed regression equation) was subtracted from the initial insect weights.

The insects were each provided with a dish of artificial diet (approximately 1.5 g of the P28% diets or 2.5 g of the P14% diets, since greater quantities of the latter were eaten) which had been oven dried (24 h at 35°C), weighed to within 1 mg and allowed to equilibrate to ambient RH for 24 h. The dishes, designed to prevent spillage of the diets, each consisted of a small petri dish (5.5 cm) glued to the center of a larger one (9.0 cm), thus forming a central dish surrounded by a moat. Covering the central dish containing the diet was a plastic lid with four 1.5 cm holes through which the insects could eat. To provide ventilation and prevent the hygroscopic diets from becoming moist, the plastic boxes had a 1.5 cm hole drilled in their lids.

At the beginning of each subsequent light phase (i.e., at 24-h intervals), the diets were removed, replaced by fresh diet, and later dried and re-weighed to determine amounts eaten. The frass produced by each insect was collected daily and stored separately in a freezer. This was later dried, weighed, ground to a fine powder and mixed thoroughly before samples were analyzed for nitrogen,

carbohydrate and tannic acid (see below).

Stadium duration was recorded to within 12 h. Newly molted insects were killed using CO_2. A sample of these (21 insects) was oven dried (48 h at 80°C), weighed and stored in a freezer for nitrogen analysis. The remaining insects (25; two died at an early stage in the experiment) were dissected immediately after death and their guts preserved in aqueous Bouins fluid for later histological analysis. Of these, seven were spoiled during the preservation period, leaving 18 for histological examination.

To obtain a regression for the estimation of dry weights at the start of Day 1, a separate experiment was performed in which 20 newly-molted fifth instars were treated up to the beginning of Day 1 in the same manner described above for the insects in the main experiment. They were then weighed and deprived of food (but not water) until the gut had cleared, killed, dried in an oven and re-weighed. The faeces were collected, oven dried and weighed to correct for residual food in the gut as described above. These same insects were used to obtain an estimate of nitrogen content of the insects at the start of the experiment.

Chemical analyses

Soluble carbohydrate in the frass was determined using the anthrone method described by Deriaz (1961). A mixture of equal concentrations of dextrin and sucrose was used to

construct standard curves, since these carbohydrates occurred at equal concentrations in the diets.

Nitrogen levels in the insects, the frass and the diets were determined using the microkjeldahl method. The factor 15.8%, as obtained from an analysis of the diets, was used to convert dietary protein levels to nitrogen levels for use in the calculation of nutritional indices for nitrogen. The spectrophotometric Folin-Denis method (Allen et al. 1974) was used to determine levels of tannic acid excreted in the frass. The Folin-Denis reagent gave positive results for frass from control insects, which had had no dietary source of tannins, producing an optical density equivalent to 30.7 and 15.7% tannic acid for faeces from insects fed 28 and 14% protein diets, respectively. This meant that no reliable estimates could be made of the tannic acid content of frass from individual insects, since not only would the measures vary with tannic acid content but also with the levels of interfering substances in the frass. A rough estimate of the tannic acid content of frass was therefore calculated as the difference in optical density between means for control and tannin-fed samples for each of the four diets; this yielded estimates of 8.1 and 8.8% for insects fed the 28 and 14% protein diets, respectively. The intefering substances may include phenolic amino acids and ascorbic acid (Hagerman and Butler 1989). It seems that in this case phenolic amino acids may have made a major contribution to the interference

with the Folin-Denis test since, as indicated above, the levels of interference were proportional to the protein content of the diets. This illustrates that caution is needed in interpreting results obtained when using the Folin-Denis method for the analysis of ecological materials.

Calculation of nutritional indices

All weights referred to are dry weights.

Growth [GT; GN]:

Total growth (GT) = wt. of insect+exuviae after final ecdysis - estimated weight on Day 1 (mg)

Nitrogen growth (GN) = Nitrogen eaten - total nitrogen in frass

Consumption [CT; CP; CC]:

Total consumption (CT) = consumption (Day 1+Day 2+ .... +final day) (mg)

Consumption of protein (CP) = CT x proportion protein in diet (mg)

Consumption of carbohydrate (CC) = CT x proportion carbohydrate in diet (mg)

Approximate digestibility [AD; AD(C)]:

Approximate digestibility (AD) = (nutrients ingested - nutrients in frass)/(nutrients ingested)

Approximate digestibility of carbohydrates (AD(C)) = (carbohydrates ingested - carbohydrate in frass)/ (carbohydrates ingested)

Efficiency of conversion of ingested food [ECI; ECI(N)]:

Efficiency of conversion of ingested nutrients (ECI) = GT/nutrients ingested

Efficiency of conversion of ingested nitrogen (ECI(N)) = GN/nitrogen ingested

Efficiency of conversion of digested food [ECD]:

ECD = GT/(nutrients ingested - nutrients in frass)

Frass nitrogen levels were not corrected for uric acid (Bhattacharya and Waldbauer 1972), so that AD will be slightly underestimated in the results presented. When no correction is made for faecal uric acid, GN can be calculated as the difference between the nitrogen ingested and the nitrogen in the frass (unassimilated + excretory; see Waldbauer 1968). This was favored over the alternative measure (nitrogen in the carcass at the end of the experiment minus estimated nitrogen at the beginning of the experiment) since it avoids the use of an estimate of initial nitrogen levels and thus provides a more direct measure. Additionally, the sample size was restricted for measurement of nitrogen levels in the carcasses, since a subsample of insects was used for histological analysis (see above). However, there is a possible source of inaccuracy in estimating GN from frass nitrogen, since volatile nitrogen compounds may be lost from the frass prior to analysis (Schroeder 1976, Fox and Macauley 1977). If this were the case, GN estimated from frass nitrogen would be significantly higher than the estimate of GN obtained from direct measurement of nitrogen in the carcasses. The means

using the frass and carcass measurements were 21.27 mg and 20.57 mg, respectively; these did not differ significantly using a t-test for matched pairs ($t = 1.12$ with 20 df; $P>0.28$).

AD, ECI and ECD are measures of the utilization of nutrient substances. Cellulose, which is not digestible by locusts (Dadd 1960a), was therefore not included in the calculation of these indices (see Simpson and Simpson 1990). Similarly, the tannic acid was added to the diets after they had been prepared to contain 14 or 28% of proteins and carbohydrates (see Chapter 1) and thus further diluted the nutrient concentrations. It was therefore necessary in calculating amounts of carbohydrates and protein eaten to correct for this dilution by tannic acid. However, caution is needed in deciding on the extent of any such adjustment, since agitation of the diet dishes by the insects during the experiment may cause some of the tannic acid to settle at the bottom of the feeding dishes. In extreme cases, this may leave an effective concentration of 7.5% tannic acid in the diets (Chapter 1). All nutritional indices were therefore calculated twice, assuming ingestion of 10% and of 7.5% tannic acid. In no case did this make a difference to statistical outcome. The results presented here have been corrected assuming the ingestion of 7.5% tannic acid.

Since locusts compensate for the dilution of protein by increased consumption, and the addition of tannic acid to

the test diets was not balanced by the addition of cellulose to the control diets (see Chapter 1), it was necessary in calculating amounts eaten to adjust for this factor. There was no qualitative difference in statistical outcome between uncorrected amount eaten and that corrected for the expected compensatory increase in feeding due to dilution by 7.5% or 10% tannic acid. Results presented here are for correction at the 7.5% level.

It was not possible to test for an effect of tannic acid on AD or ECD, since the levels of tannic acid in the frass of individual insects could not be measured reliably (see "Chemical analyses") and would therefore introduce a systematic bias in estimating total excreted nutrients [=dry wt. of frass - (cellulose eaten + tannic acid in faeces)]. Carbohydrates and nitrogen were, however, measured directly in the frass and it was therefore possible to test for effects of tannic acid on AD and ECD of these nutrient categories.

Histology

Midgut sections (approximately 5 mm, taken from the area posterior to the junction of the caeca and anterior to the junction of the malpighian tubules with the alimentary canal) were removed from the aqueous Bouins fluid in which they had been preserved and passed through a series of ethanol and xylene washes for de-hydration before embedding

in paraffin wax. Sections (30 - 40 from each specimen, 10um thick) were stained using hematoxylin and counterstained with eosin before mounting. Unlike Lepidoptera, the midgut of Orthoptera is undifferentiated (Dow 1986) and it was therefore not considered crucial that the midgut of each insect was sampled from precisely the same region.

Statistical analyses

Statistical analyses were performed using the SPSS-X (SPSS Inc.) mainframe statistical package. To test the effects of protein, carbohydrate and tannic acid levels on consumption (CT, CN and CC) and GN, ANCOVA was used with four factors each having two levels [tannic acid (10% or absent), protein and carbohydrate (each 14% or 28%) and sex] and, for all tests except ECI(N), the natural logarithm of insect weight on Day 1 as a covariate. The same design was used to test GT but, owing to the limited sample size, sex was excluded as a factor. Duration of instar differed little between insects (grand mean $\pm$ S.E.$_{DAYS}$ = 11.7 $\pm$ 0.06) and there was no detectable pattern corresponding to any of the treatments; this variable was therefore not included as a covariate in the statistical models. Parallel slopes were verified for all ANCOVAs ($P>0.1$). ANOVA was used to test AD(C), ECI, ECI(N) and ECD. In all cases, f-ratios and significance levels presented here (Table 1) were generated using full factorial models, since this provides a more rigorous test

of the hypotheses of interest.

The conventionally used RGR (GT/average insect weight x duration of instar) and RCR (CT/average insect weight x duration of instar) (Waldbauer 1968) were abandoned in favor of the analysis of covariance for theoretical reasons concerning the use of ratios in parametric statistical analyses (see Chapter 5). Additionally, consumption and growth data were corrected for initial insect weight, rather than the conventionally used average larval weight (= [initial wt + final wt]/2; Waldbauer 1968), since the latter corrects the data for treatment effects on insect growth (see also Farrar et al. 1989). In the case of consumption, this may result in an underestimate of the differences in amounts eaten of different diets. This is because, while designed to correct the data for any effects of insect weight on amounts eaten, it also "corrects" the data for any effects of amounts eaten on insect growth. In the case of growth rates, growth is itself a component of average larval weight and this index therefore simply corrects the data for the treatment effect under investigation.

All data were tested for homoscedasticity using Bartlett's test, and for normality using the Kolmogorov-Smirnov test. In all cases the Kolmogorov-Smirnov test was satisfied ($p>0.05$). Data for ECI, ECD and AD were heteroscedastic ($p<0.05$). Data for ECI and ECD were therefore transformed prior to statistical analysis using

the natural logarithmic transformation. The Fisher-Behrens test, which has no assumption of equal variances (Campbell 1974), was used to test protein and carbohydrate as main effects on AD, since no suitable transformation could be found for this variable.

RESULTS

Table 1 contains a summary of f-ratios for the results presented below, and Tables 2 and 3 contain the means collapsed over main effects and two-way interactions, respectively. There were no significant three-way or higher order interactions, and these have therefore been omitted from Tables 1 and 3.

Growth (GT and GN)

A significant tannin x protein interaction (P=0.002) indicated that tannic acid reduced total growth (GT), but only of those insects fed P14% diets (Tables 1 and 3). Additionally, nitrogen growth (GN) was significantly lower both on the P14% diets (P<0.001) and the diets containing tannic acid (P<0.001). From this it appears that the effect of tannic acid on GT can be accounted for, partially at least, by decreased GN.

While low protein levels resulted in decreased GN, the opposite effect was observed for GT (P<0.006). Therefore,

Table 1. Table of f-ratios from the analysis of variance for the effects of protein, carbohydrate and tannic acid levels on consumption and utilization of artificial diets by *Locusta migratoria* nymphs. *=P<0.05; **=P<0.01; ***=P<0.001; { }=means for which valid comparisons could not be made (see text for further details).

Source	df	F-values									
		Total growth (GT)	Nitrogen growth (GN)	Total consumption (CT)	Consumption of protein (CP)	Consumption of carbohydrate (CC)	Approximate digestibility of carbohydrate (AD(C))	Efficiency of conversion of ingested food (ECI)	Efficiency of conversion of ingested nitrogen (ECI(N))	Efficiency of conversion of digested food (ECD)	Percentage ingested nitrogen in frass
Covariate											
ln(Insect weight)	1	4.39(P=0.06)	2.64	5.33*	7.64*	3.19	-	-	-	-	-
Main effects											
Tannic acid (T)	1	4.34(P=0.06)	17.42***	5.05*	5.99*	5.38*	3.14(P=0.08)	12.73**	9.39**	{12.58**}	9.41**
Protein (P)	1	10.80**	34.89***	107.89***	48.97***	101.56***	0.32	0.33	1.36	0.47	1.36
Carbohydrate (C)	1	6.79*	0.57	20.05***	28.05***	128.25***	3.44(P=0.08)	0.19	44.94***	1.93	45.10***
Sex (S)	1	-	0.48	<0.00	0.03	0.30	-	-	0.03	-	0.03
Two-way interactions											
T x P	1	15.67**	2.28	5.70*	5.01*	4.23*	0.03	0.46	0.01	0.41	0.01
T x C	1	0.39	0.75	0.58	0.31	<0.00	0.89	0.12	0.32	0.06	0.32
C x P	1	<0.00	0.55	1.71	0.07	4.70*	0.39	0.58	3.83(P=0.06)	0.56	3.84(P=0.06)
S x P	1	-	-	4.49*	0.92	5.19*	-	-	0.03	-	1.94
df Residual		12	29	29	29	29	21	13	30	13	30
df Total		20	45	45	45	45	29	20	45	20	45

Table 2. Cell means for the main effects of dietary protein, digestible carbohydrate and tannic acid levels on consumption and utilization of artificial diets by *Locusta migratoria* nymphs. Symbols are as in Table 1.

Factor	level	Means										
		Total growth (GT) mg	Nitrogen growth (GN) mg	Total consumption (CT) mg	Consumption of protein (CP) mg	Consumption of carbohyd-rate (CC) mg	Approximate digestibility (AD)	Approximate digestibility of carbohy-drate (AD(C))	Efficiency of conversion of ingested food (ECI)	Efficiency of conversion of ingested nitrogen (ECI(N))	Efficiency of conversion of digested food (ECD)	Percentage ingested nitrogen in frass
Tannic acid	0%	177.6[P=0.06]	22.8***	1276.3*	223.4*	248.9*	(0.833	0.965[P=0.08]	0.316**	0.648*	(0.380**	35.2**
	10%	160.2	19.9	1151.6	210.7	218.2	0.807)	0.938	0.384	0.599	0.477)	40.1
Protein	28%	150.0**	23.5***	923.6***	242.1***	182.0***	0.795*	0.949	0.352	0.614	0.449	38.6
	14%	187.0	19.2	1474.9	193.5	279.6	0.843	0.955	0.358	0.630	0.421	37.0
Carbohydrate	28%	176.6*	21.1	1081.8***	197.8***	283.8***	0.853**	0.968[P=0.08]	0.353	0.672***	0.419	32.8***
	14%	155.7	21.5	1352.4	237.4	177.4	0.780	0.937	0.358	0.569	0.458	43.1

Table 3. Cell means for the two-way interactive effects of dietary protein, digestible carbohydrate and tannic acid levels on consumption and utilization of artificial diets by *Locusta migratoria* nymphs. Significance levels refer to the significance of two-way interactions; symbols used are as in Table 1.

Diets	Means										
	Total growth (GT) mg	Nitrogen growth (GN) mg	Total consumption (CT) mg	Consumption of protein (CP) mg	Consumption of carbohydrate (CC) mg	Approximate digestibility (AD)	Approximate digestibility of carbohydrate (AD(C))	Efficiency of conversion of ingested food (ECI)	Efficiency of conversion of ingested nitrogen (ECI(N))	Efficiency of conversion of digested food (ECD)	Percentage ingested nitrogen in frass
T0%-P28%	138.2**	24.6	912.8*	241.1*	188.7*	{0.814	0.963	0.306	0.643	{0.379	35.7
T0%-P14%	226.8	21.3	1579.2	208.6	299.1	0.850	0.967	0.328	0.652	0.381	34.8
T10%-P28%	159.9	22.6	932.6	243.0	176.4	0.778	0.932	0.390	0.589	0.506	41.1
T10%-P14%	160.5	17.2	1370.6	178.5	260.0	0.836}	0.943	0.378	0.608	0.448}	39.2
T0%-C28	183.7	22.3	1115.4	204.1	294.6	{0.867	0.984	0.308	0.690	{0.361	31.0
T0%-C14%	170.0	23.3	1469.3	246.5	194.1	0.788	0.944	0.325	0.598	0.403	40.2
T10%-C28%	171.6	19.8	1048.3	191.5	273.0	0.840	0.947	0.384	0.653	0.460	34.7
T10%-C14%	144.2	19.9	1255.0	229.9	163.4	0.774}	0.932	0.384	0.545	0.448}	45.5
C28%-P28%	161.1	23.1	852.0[P=0.09]	223.5	223.5*	0.818	0.962	0.353	0.648[P=0.06]	0.436	35.2[P=0.06]
C28%-P14%	192.2	19.1	1311.7	172.1	344.2	0.889	0.973	0.352	0.695	0.401	30.5
C14%-P28%	136.8	24.0	1009.6	264.4	132.2	0.767	0.937	0.350	0.572	0.464	42.8
C14%-P14%	179.2	19.4	1638.1	215.0	215.0	0.792	0.938	0.368	0.566	0.451	43.4
MALE-P28%	-	21.7	865.7*	226.9	169.9*	0.790	-	-	0.603	-	39.7
MALE-P14%	-	17.6	1321.8	173.4	247.3	0.854	-	-	0.642	-	35.8
FEMALE-P28%	-	25.3	981.6	257.3	194.1	0.799	-	-	0.624	-	37.6
FEMALE-P14%	-	20.9	1627.9	213.6	311.8	0.833	-	-	0.619	-	38.1

the increase in total growth associated with low protein diets could not be accounted for by increased nitrogen growth. This suggests that the accumulation of non-nitrogenous substances, probably carbohydrates and lipids, was restricted on the diets containing high levels of protein or excessive on diets with low protein levels.

Consumption (CT, CN and CC)

Figure 1 provides a graphical presentation of the influence of proteins, carbohydrates and tannic acid on the amounts of protein and carbohydrates eaten. In the figure, the main-effect means (adjoining the vertical axis) are progressively expanded into their constituent sub-means. In what follows I will consider the vertical length of lines joining two points the same unless there is statistical reason to conclude otherwise. For example, the line joining points (c) and (d) appears in the figure to be longer than that joining (g) and (h). This is however not statistically so, since there was no significant protein x carbohydrate x tannic acid three-way interaction.

Carbohydrates, proteins and C x P interactions: Low levels of proteins ($P<0.001$) as well as of carbohydrates ($P<0.001$) resulted in increased consumption of diet (CT), indicating a compensatory feeding response for both nutrients (Tables 1 and 2). This is evident in Fig. 1 since, if compensation

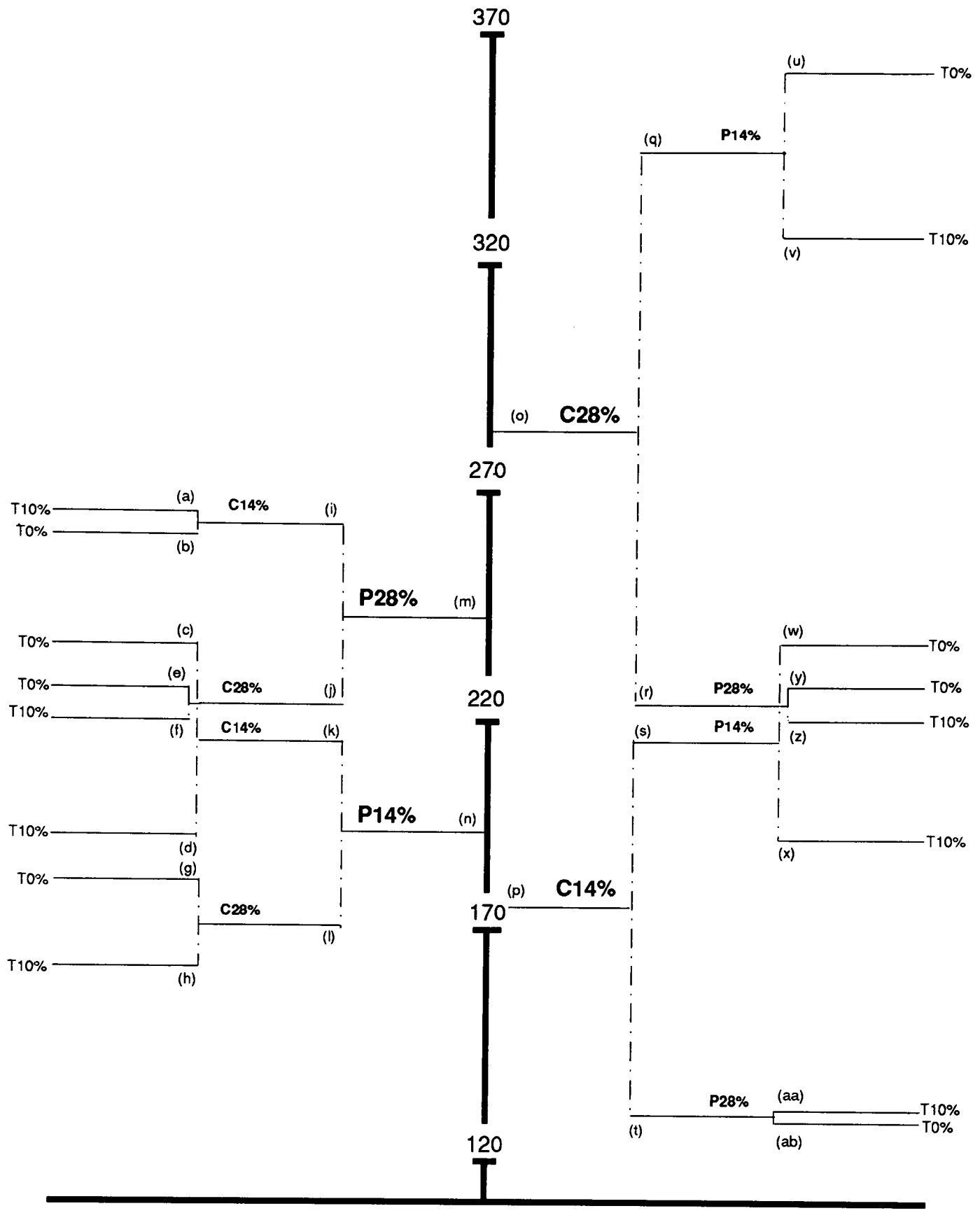


Figure 1. Graphical representation of amounts of protein and digestible carbohydrate ingested by *Locusta migratoria* over the course of the fifth instar. The insects were fed one of 8 artificial diets varying in tannic acid (10% or absent), protein and digestible carbohydrate levels (14 or 28%). The outermost points each represent the mean levels of protein (left) and carbohydrate (right) ingested for a single diet (e.g., point (u) represents the mean level of carbohydrate ingested by 8 insects fed the C28%-P14%-T0% diet, and point (g) represents the amount of protein ingested by the same insects). These means are progressively collapsed over tannic acid (e.g., points (q) and (l)) and then protein (e.g. point (o)) or carbohydrate (e.g., point (n)) levels. Points (m) and (n) adjoining the vertical axis therefore represent the mean amount of protein ingested on all high and low protein diets respectively, and points (o) and (p) represent the mean amounts of carbohydrate ingested on all high and low carbohydrate diets. Table 1 contains statistical analysis of the means presented here. See text for further discussion.

for neither proteins nor carbohydrates were taking place, the point (n) on the figure would be half the value on the vertical axis as (m) and the point (p) would be half the value of (o).

It is, however, clear that compensation for both nutrients was incomplete, since the insects fed P14% and C14% diets had significantly lower protein consumption (CP) ($P<0.001$) and carbohydrate consumption (CC) ($P<0.001$), respectively, than those fed diets containing these nutrients at the 28% level (Tables 1 and 2). If compensation for proteins was complete and there was no compensation for carbohydrates, the point (n) in Fig. 1 would be at the same level as (m), and this level would be determined by the mechanisms regulating protein intake. Likewise, if there was complete compensation for carbohydrates and no compensation for proteins, points (p) and (o) would be at the same level. This was clearly not the case, and consideration of the regulation of intake must therefore take into account the simultaneous influence of both nutrient groups. That the distance separating point (m) from (n) is smaller than that between points (o) and (p), indicates that consumption was regulated more strongly around protein than carbohydrate intake.

High protein levels resulted in reduced CC relative to low protein levels ($P<0.001$). Similarly, there was lower protein consumption on high than low carbohydrate diets

($P<0.001$) (Tables 1 and 2). There are two, non mutually exclusive, explanations for this. Firstly, it is likely that high levels in the diets of either nutrient had the effect of restricting intake of the other. Secondly, compensation for low levels of either nutrient had the effect of increasing, inadvertently, intake of the other. These are important consequences of dietary imbalance, and in what follows I will refer to the former situation as *incidental restriction of intake*, and the latter as *incidental augmentation of intake*; Fig 2 presents a schematized diagram representing these concepts. Therefore, intake of a nutrient is said to be 'incidentally restricted' if regulation for one or more other nutrients results in the cessation of feeding at a point when the first nutrient has not yet been ingested in the quantities it would if intake were regulated for that nutrient alone (case 3 in Fig. 2). When intake is regulated to increase the levels ingested of a limiting nutrient and this results in the ingestion of an excess of a second nutrient, then 'incidental augmentation' of intake occurs (case 2 in Fig. 2). 'Incidental' is used since the restriction and augmentation of intake are incidental to the mechanisms regulating intake of the nutrient under consideration; intake is determined by the mechanisms regulating consumption of other nutrients.

From the foregoing, it is clear that the amounts of carbohydrates eaten were influenced by both carbohydrate and

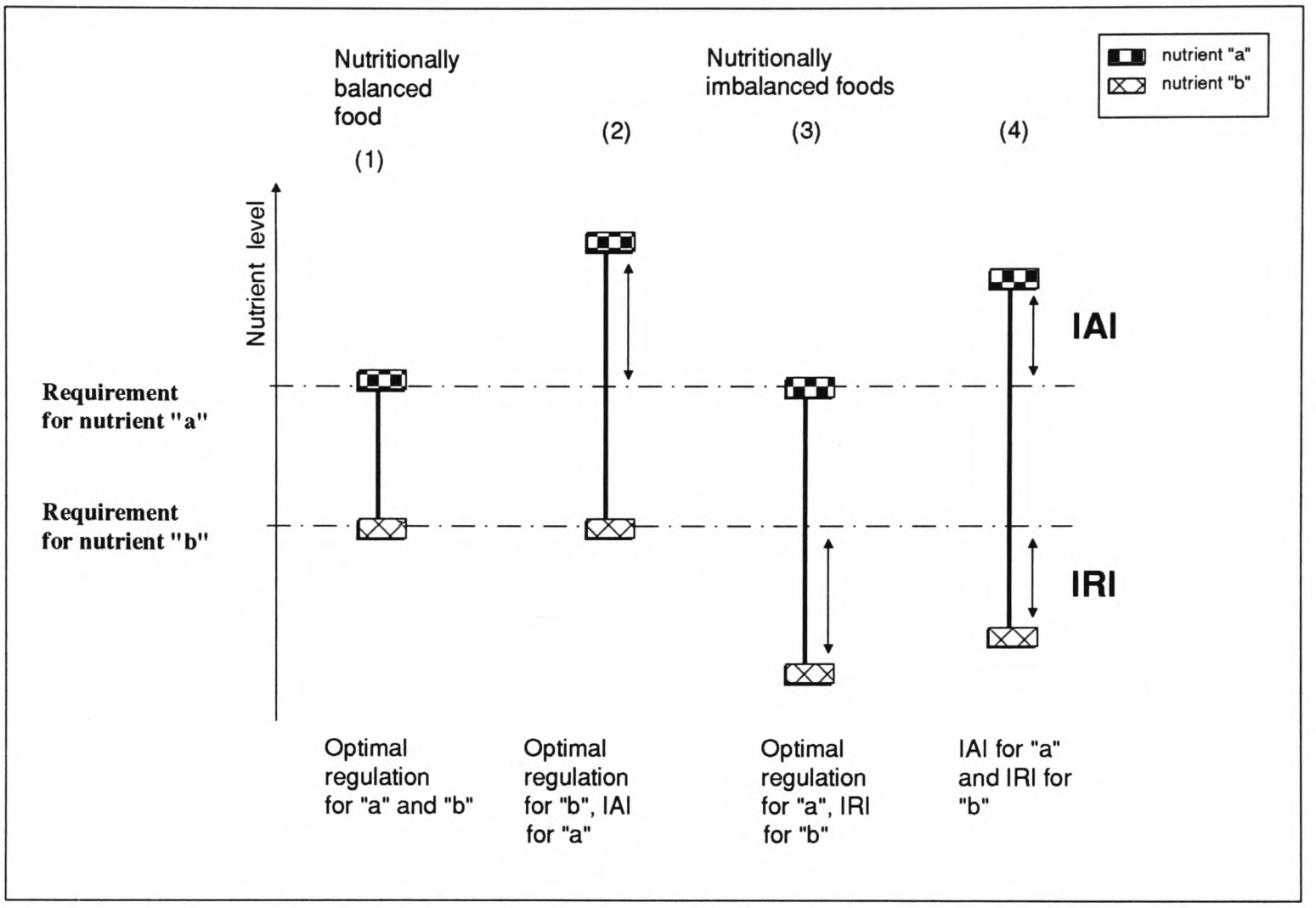


Figure 2. Diagram to illustrate incidental augmentation of intake (IAI) and incidental restriction of intake (IRI). The checkered and cross-hatched blocks represent different nutrients within a single food, and the broken horizontal lines the requirement of an hypothetical animal for each. Solid bars separating the two nutrients depict the proportion by which the nutrients differ in the food.

protein levels. In addition, a significant carbohydrate x protein interaction (P=0.039) (Table 1) suggests that the effects of carbohydrate and protein levels on CC were not independent of each other. Comparison of the means for this interaction (Table 3) reveals that protein levels had a larger effect on carbohydrate consumption when carbohydrate levels in the diet were high (C28% diets) than when they were low (C14% diets). This can be seen in Fig. 1 by comparing the length of the line joining points (q) and (r) with that of the line joining (s) and (t).

The direction of this interaction provides an interesting indicator as to the relative importance of incidental augmentation and restriction of intake in explaining the influence of protein levels on carbohydrate consumption. Incidental augmentation of carbohydrate intake would be most acute on the C28-P14% diets (point (q) in Fig. 1), because on these diets the insects ate more to compensate for low protein levels, and for every unit of protein ingested two units of carbohydrate were eaten. Therefore, incidental augmentation of carbohydrate intake will affect the shape of Fig. 1 predominantly by extending the length of line (r)-(q) in the direction of (q), and hence by raising (o) (the mean of (r) and (q)). Incidental restriction of carbohydrate intake, on the other hand, will be most acute for the C14-P28% diets (point (t)) because here the insects, while compensating for carbohydrates, were forced to ingest high

levels of protein. The effect of incidental restriction of intake will therefore be to constrain compensation for carbohydrates, extending the line (s)-(t) in the direction of (t), thereby limiting the approach of (p) towards (o). Since the significant carbohydrate x protein interaction for CC is based on the fact that line (r)-(q) is longer than (s)-(t), this therefore suggests that incidental augmentation of carbohydrate intake was the major factor accounting for the influence of protein levels on CC. However, incidental restriction of intake also played a role, and the extent to which this is true is indicated by the length of line (s)-(t).

Interestingly, there was no significant protein x carbohydrate interaction for CP (Table 1). This suggests that carbohydrate levels influenced protein consumption equally through incidental restriction and augmentation of intake. In Fig. 1, this can be seen in the similar lengths of lines (i)-(j) and (k)-(l).

Tannic acid and tannin x nutrient interactions: As a main effect, tannic acid resulted in a significant decrease in CT (P=0.032), and hence CP (P=0.021) and CC (P=0.028) (Tables 1 and 2). A significant tannin x protein interaction for CT (P=0.024; Table 1) indicated that the decreased consumption occurred only when the diets containing tannic acid simultaneously had low levels of protein (see the means

for this interaction in Table 3). This suggests that the effect of tannic acid on CT was through the restriction of compensation for low levels of dietary protein. Confirmation for this can be found in the cell means from the significant tannic acid x protein interaction for CP (P=0.033) (Tables 1 and 3). These verify that, overall, compensation for proteins was more complete in the absence of tannic acid (86.5%) than when tannic acid was present in the diets (73.5%), where efficiency of compensation is calculated as [(CP on P14% diets/CP on P28% diets) x 100%] - a reduction in compensation of 13 percentage points due to tannic acid. In Fig. 1, this can be seen by comparing the level of point (c) with point (d), and point (g) with point (h). A comparison of the combined lengths of lines (a)-(b) and (e)-(f) with (c)-(d) and (g)-(h) illustrates the extent to which tannic acid had a more pronounced effect on consumption of the P14% than the P28% diets.

There was, in addition, a significant tannin x protein interaction for CC (P=0.049). From the cell means for this interaction (Table 3) it can be seen that the effect of tannic acid was to restrict the increased intake of carbohydrates on the P14% diets. The difference of the means for CC when tannic acid was present or absent in the P28% diets was 12.3 mg, while the same comparison across the P14% diets reveals a difference of 39.1 mg. In Fig. 1, this can be seen in the greater lengths of lines (u)-(v) and (w)-(x)

than (y)-(z) and (aa)-(ab). In terms of the terminology introduced above, tannic acid therefore reduced CC by limiting incidental augmentation of carbohydrate intake (point (v)), as well as through restricting the intake of carbohydrates directly (point (x)).

Unlike the significant tannin x protein interaction for CP mentioned above, there was no significant tannin x carbohydrate interaction for CC - i.e., tannins did not restrict compensation for carbohydrates. In Fig. 1, this can be seen in the similarity of the combined length of lines (u)-(v) + (y)-(z) to that of (w)-(x) + (aa)-(ab). Compare this to the situation for proteins, in which the combined length of (c)-(d) + (g)-(h) is appreciably greater than that of (a)-(b) + (e)-(f).

Likewise, there were no significant tannin x carbohydrate interactions for CP or CT. It is thus apparent that, whereas tannic acid interacted with protein levels in influencing consumption (CT, CP and CC), there was no such tendency for tannic acid to interact with carbohydrate levels. In other words, tannic acid had the effect of restricting compensatory feeding for proteins, but not for carbohydrates. This difference can be explained by the fact that compensation for proteins was more complete than for carbohydrates, resulting in higher cumulative levels of tannic acid being ingested in the P-14% than the C-14% diets. Thus, a threshold is crossed in the cumulative intake of

tannic acid beyond which consumption is reduced. This threshold exists at some level of dietary intake between that for the T0%-C14% (grand mean = 1469.3 mg/5th stadium) and the T0%-P14% (1579.2 mg/5th stadium) diets.

Protein x sex interaction: Tables 1 and 3 show a significant ($P<0.05$) sex x protein interaction for CT, such that the ratio of P14%-diets/P28%-diets eaten was higher for females than for males. There was in addition a significant ($P<0.05$) sex x protein interaction for CC, but not for CP. Perhaps, therefore, males and females have different points around which consumption of carbohydrates and/or proteins are regulated. Any such differences in nutritional requirements would not be surprising considering that the fifth instar is a period of sexual development in *L. migratoria*.

Histopathological effects of tannic acid

The insects subjected to histological examination were categorized into those with and without signs of damage to the midgut tissues. An insect was classified as damaged if one or more areas of epithelial degeneration were recognized (Bernays 1978, Bernays et al. 1980, Steinly and Berenbaum 1985). Of the tannin-fed insects, 90% showed signs of histological damage, while in only 33% of the controls was some form of lesion detected ($P=0.009$ using Fisher's Exact Test; Norusis (1988)). Bernays (1978) found that 45% of *L.*

migratoria fed tannic acid (8-28%) applied to wheat leaves developed lesions in the midgut and caecum, while 10-12% of the controls showed signs of damage.

Utilization efficiencies (AD, AD(C), ECI, ECI(N), ECD)

Digestibility: Approximate digestibility of total nutrients (AD) was significantly higher on the C28% than the C14% diets ($d_{(23,20)}=4.30$; $p<0.01$ - Fisher-Behrens test), and lower on the P28% than the P14% diets ($d_{(21,22)}=2.56$; $p<0.05$) (see Table 2 for the means). There was, additionally, a suggestion that the digestibility of carbohydrates (AD(C)) was higher on the C28% diets, but this was statistically non-significant (P=0.078).

As explained in the Methods section, it was not possible to test for an effect of tannic acid on AD. However, since carbohydrates were measured directly in the frass, the effect of tannic acid on AD(C) could be determined. There was a suggestion that AD(C) was lower for tannic acid-fed insects (P=0.079). This may be due to the presence in the frass of glucose, a breakdown products of tannic acid (Bernays 1978, Bernays and Chamberlain 1980) rather than some direct effect of tannic acid on the digestion of carbohydrates. Consistent with this possibility, is the fact that the mean level of tannic acid recovered was 8.45% of the frass weight, as compared with an expected recovery of approximately 12.1% if all the ingested tannic acid were

recovered in the frass.

Conversion of ingested food: The efficiency of conversion of nitrogen (ECI(N)) was reduced for the insects fed tannic acid (P=0.005), an observation consistent with the increased proportion of ingested nitrogen found in the frass (Table 2). Tannin-fed insects had a higher occurrence of lesions in the midgut epithelium than the controls, and Bernays et al. (1980) have observed associated with such damage in *L. migratoria* the sloughing into the lumen of caecal and midgut epithelia. Such sloughing and replacement of damaged cells from the midgut epithelium may be a common feature among herbivorous insects (Dow 1986). It is possible that in the present study the egestion of debris resulting from cell damage accounted for the increased levels of nitrogen in the frass of the tannin-fed insects, and that this was not directly observed because the insects were examined after the final moult when the midguts were empty. Bernays et al. (1980) used higher levels of tannic acid (20-23%), which had the result that the insects they examined were not newly molted adults, but had often died from the treatment during the larval stadia. It is also possible that higher levels of nitrogen in the frass of tannin-fed insects were due to increased secretion of peritrophic membrane, which in some insects may contain up to 55% protein (Chapman 1985a).

Unlike ECI(N), conversion of total food ingested (ECI)

was higher for the tannin-fed insects than the controls (P=0.004; Tables 1 and 2). Since the higher ECI of the tannin-fed insects could not be accounted for by conversion of nitrogen, this indicates that the efficiency of conversion to growth of non-nitrogenous compounds was increased by the presence of tannic acid in the diets. It may be that increased ECI on the tannic acid diets was primarily related to decreased consumption of these diets; a negative relationship between levels of consumption and ECI has frequently been observed in herbivorous insects (see Discussion). A further possibility is that glucose, a breakdown product of tannic acid, was utilized by the tannin-fed insects (Bernays 1978, Bernays and Chamberlain 1980, Bernays et al. 1980).

While protein was not significant as a main effect on ECI(N), there was a suggestion of a carbohydrate x protein interaction (P=0.06) in which the conversion of nitrogen was particularly high for the C28-P14% diets (Tables 1 and 3). The C28-P14% diets were the diets on which the least protein was consumed (see point (1) in Fig. 1). Additionally, ECI(N) was significantly higher on the C28% diets (P<0.001), and these were also the diets on which there was incidental restriction of protein intake (Tables 1 and 3; Fig. 1). The proportion of ingested nitrogen excreted in the frass was significantly higher on the C14% than the C28% diets.

It thus appears that there was, in general, a negative

relationship between the amount of both nitrogen and non-nitrogen based nutrients ingested and the efficiency with which ingested food was utilized for growth. However, this relationship was not simple, and it appears that an increase in ECI(N) was dependant on the simultaneous ingestion of high levels of carbohydrates.

DISCUSSION

Following the early debate regarding the relative importance of allelochemicals vs. nutrients in host selection by phytophagous insects (Dethier 1954, Fraenkel 1959, Fraenkel 1969, Thorsteinson 1960, House 1961, Beck 1965, Kennedy 1965, Cartier 1968), there has been a tremendous proliferation of publications dealing with both aspects of insect/plant interactions (e.g. see Bernays 1985, Slansky and Scriber 1985, Rosenthal 1986). While both fields have independently produced appreciable advances in the understanding of herbivorous insects, it is only recently that studies on the interactions that may occur between nutrients and allelochemicals have begun to appear in the literature (e.g., Lincoln et al. 1982, Duffey et al. 1986, Hare 1987, Broadway and Duffey 1988, Johnson and Bentley 1988, Bloem et al. 1989, Bloem and Duffey 1990, Raubenheimer and Simpson 1990).

There has, likewise, been scant attention paid to the

interactions that occur between nutrients when insects eat nutritionally imbalanced diets. Sang (1959), using axenically cultured *Drosophila* larvae, was the first to investigate in detail the effects on insect development of different ratios of nutrients (proteins and vitamins) in artificial diets. House (1965) extended this approach to the study of a phytophagous insect, *Celerio euphorbiae*, and later to the fly *Agria affinis* (House 1966a). In the more than two decades following the publications of House, there have been few studies explicitly addressing the question of nutrient imbalance. The majority of these have investigated the specific question of balance of amino acids in proteins (Manoukas 1981, Horie and Watanabe 1983, Karowe and Martin 1989). While amino acid imbalances may in some respects be viewed to have special significance among nutritional imbalances (Harper 1964), it is equally important to understand imbalances among different nutrient groups.

The scant attention paid to the subject of dietary imbalance has not been wholly for want of adequate precedents, since the work of Sang (1959, 1962), Gordon (1959) and House (1965, 1966a, 1966b, 1969) had illustrated the potential importance of nutritional interactions. Perhaps this gap can be attributed partially to the methodological and interpretive problems arising from studies in which more than one dietary component are systematically and simultaneously varied. For example, there

are severe limitations in the number of chemical variables that can be measured and experimentally manipulated in the use of plant tissue, while the use of artificial diets may in many instances represent an unrealistic over-simplification. A sound compromise, initially at least, is therefore to introduce controlled complexity into the use of artificial diets. Factorial experimental designs of the type reported here are well suited to such studies.

Results of the present work have demonstrated some ways in which protein levels may interact with both carbohydrates and tannic acid, affecting feeding behavior and physiology of *L. migratoria* in a significant and sometimes complex way. However, I found no evidence of three-way statistical interactions, in which the effects of tannic acid, protein or carbohydrate depended on the combination of the levels in the diets of the other two factors.

Compensatory feeding for carbohydrates and proteins

It is well known that in a no-choice assay *L. migratoria* nymphs regulate intake with respect to dietary protein levels (Dadd 1960a, Simpson and Abisgold 1985, Chapter 1), but the same has not hitherto been conclusively demonstrated for carbohydrates. Several insect species are now known to increase consumption in response to decreased dietary carbohydrate levels, including cockroaches (Gordon 1968, Bignell 1978), female mosquitoes (Nayar and Sauerman 1974),

blowflies (Dethier et al. 1956, Gelperin and Dethier 1967, Simpson et al. 1989), Mediterranean fruit flies (Nestel et al. 1985) and the butterfly *Pieris brassicae* (David and Gardener 1961). Simpson and Abisgold (1985) stressed that the failure of their 12-h experiments to detect compensatory feeding for carbohydrates by *L. migratoria* does not rule out the possibility that under different circumstances it might occur. More recently, I have found a significant sex x carbohydrate interaction for amounts of artificial diets eaten by *L. migratoria* nymphs over a 12-h period, and this was interpreted to indicate that only males regulated intake with respect to dietary carbohydrate levels (Chapter 1). The present study has demonstrated that, over the course of the fifth instar, both male and female *L. migratoria* increase intake when dietary carbohydrate levels are reduced from 28% to 14%.

It is nonetheless clear that in no-choice assays *L. migratoria* regulate intake more strongly around protein than carbohydrate levels, and some of the regulatory mechanisms accounting for this have now been elucidated (Abisgold and Simpson 1987, 1988, C.L. Simpson et al. 1990). Additionally, while *L. migratoria* are capable of compensating for carbohydrate deficiency by dietary selection, such compensation is more readily induced for proteins. Insects fed a protein-deficient diet then given a choice of diets containing only protein or carbohydrate selected the

protein-containing diet after a single deficient meal (Simpson et al. 1990), while it took up to 4 h for the carbohydrate-deprived insects to make the appropriate choice (Simpson et al. 1988).

That locusts should have regulatory mechanisms closely linked to protein intake is not surprising, given the extensive literature illustrating the importance of dietary nitrogen levels in insect growth, development and reproduction (Mc Neill and Southwood 1978, Mattson 1980, Scriber and Slansky 1981, Mullins and Cochran 1983). The important point to arise from the present work however, is the extent to, and manner in which other dietary components interfered with this regulatory process. A second point to note is how regulation of consumption for nitrogen intake altered the intake of other dietary components.

The effects of tannic acid

Tannic acid resulted in restricted intake of proteins, but only when the diets contained 14% protein; consumption of the 28%-protein diets was relatively unaffected by tannic acid. Rather than restricting consumption of proteins per se, it therefore appears that the effect of tannic acid was to restrict compensatory feeding for proteins. Sen Gupta and Miles (1975) found an inverse relationship between susceptibility to infestation by aphids and the ratio of phenols to amino acid nitrogen in apple leaves, and this may

represent another example of the same. Similarly, Mihaliak et al. (1987) found that camphorweed plants (*Heterotheca subaxillaris*) experimentally grown in nitrate-limiting conditions produced high levels of leaf terpenes, which prevented larvae of the soybean looper (*Pseudoplusia includens*) from increasing consumption when foliar nitrogen levels were simultaneously low. It appears that in the present study, the effect was dependent on cumulative intake since, using the same experimental regime, it was found in Chapter 1 that compensatory feeding for proteins was unaffected by the first 12 h of exposure to tannic acid.

It is interesting to speculate on the mechanisms accounting for this cumulative effect. One possibility is that insects ingesting sufficient tannic acid became ill, and were thus not capable of eating as much as the controls. Compensatory feeding would result in increased intake of tannic acid, so that any such effects would be enhanced for low-protein diets. Alternatively, rather than an unavoidable consequence of the ingestion of tannic acid, reduced consumption may have been an adaptive response to limit the adverse effects of ingesting potentially toxic compounds (Slansky and Scriber 1985). This could be a genetically fixed response to specific chemical groups, in the sense that it does not rely on the ingestion of the deterrent compound or any previous experience with it (Jermy 1986, Bernays and Chapman 1986), or a learned response. It is,

however, unlikely that a genetically fixed response can explain the present data, since any cumulative effect of a dietary component by definition involves prior experience with the chemical. A more likely explanation is that reduced consumption resulted from food aversion learning (Jermy 1986, Papaj and Prokopy 1989). In this case an animal learns to associate the chemosensory image of a toxic food with the illness resulting from its ingestion, and subsequently rejects the food. Naive *L. migratoria* given a choice between controls and diets containing 10% tannic acid tend to take their first meal on the control diets (Chapter 1), thus demonstrating that perception of this chemical does occur. Nymphs of the grasshopper *Schistocerca americana* have been shown to exhibit aversion learning when a meal of otherwise acceptable food is followed by injection with the toxic alkaloid nicotine hydrogen tartrate (Bernays and Lee 1988, Lee and Bernays 1988, 1990). Aversion learning based on nutritionally inadequate diets has also been demonstrated for *S. americana,* in relation to both the absence of suitable dietary sterols (Champagne and Bernays in prep.) and possibly protein deprivation (Chapter 3).

It is interesting that tannic acid restricted compensation for low dietary protein levels and reduced growth, in view of the expanding body of literature suggesting that the levels of nitrogen and phenolic compounds often correlate negatively in plants (del Moral

1972, Kiraly 1976, Phillips and Henshaw 1979, Miles et al. 1982, Ohmart et. al. 1985, Waring et al. 1985, Larsson et al. 1986, Glyphis and Puttick 1989, Price et al. 1989; for an exception see Horner et al. 1987). Glucosinolates (Josefsson 1970, Wolfson 1980) and terpenes (Mihaliak and Lincoln 1985, 1989, Mihaliak et al. 1987, Muzika et al. 1989) have also been found to correlate negatively with leaf nitrogen. The carbon-nutrient balance hypothesis (Bryant et al. 1983, Tuomi et al. 1984, Coley et al. 1985) has been proposed to account for this correlation from the viewpoint of resource allocation in plants. According to this, plants that occur in low-nutrient environments divert carbohydrate products of photosynthesis into the production of phenolic compounds because the nutrients necessary for their incorporation into tissue growth are limiting. By similar reasoning, plants with a low carbon/nutrient balance should produce nitrogen-based allelochemicals such as alkaloids, and there have been several reports confirming this (Rhoades and Cates 1976, Waller and Nowacki 1978, Johnson et al. 1987).

Results of the present study provide a focus on this theory at the level of the individual insect. Tannins may be more cost-effective anti-herbivore agents when produced by plants with low levels of foliar nutrients, since under these conditions compensatory feeding by herbivores would result in the ingestion of higher levels of the

allelochemicals. Sen Gupta and Miles (1975) reached a similar conclusion in their study of the chemical factors responsible for resistance of certain varieties of apple to attack by aphids. By contrast, plants which are energy- but not nutrient-limited may benefit more from the production of nitrogen-based and other allelochemicals which are more toxic and less dose-dependant than tannins.

This is not to suggest that low foliar nutrient levels have evolved in plants as a means of chemical defence; Moran and Hamilton (1980) have argued convincingly against this suggestion. Rather, I suggest that in systems where foliar nutrient levels are low as a result of constraints imposed by the environment (e.g., low nutrient soils - Rundel 1982), the production of carbon-based allelochemicals which have dose-dependant effects may be selected for. This would simultaneously influence insect population dynamics by restricting growth and fecundity (White 1984) and limit any additional damage to plants due to compensatory feeding by insects (Moran and Hamilton 1980).

There is, furthermore, evidence that the toxicity of phenolic compounds may be enhanced when protein concentrations are low. Kirkham (1954) found that phenols extracted from apple and pear leaves are strongly inhibitory to growth and sporulation of the pathogenic fungus responsible for apple scab and pear scab, but only when nitrogen levels are low. There are suggestions that this

relationship between toxicity of allelochemicals and nitrogen levels may also hold for herbivorous insects. Tomato plants produce both the glycoalkaloid *a*-tomatine and the phenolic rutin. The toxicity to *Heliothis zea* and *Spodoptera exigua* of this combination of compounds is inversely proportional to protein levels in artificial diets (Duffey et al. 1986). Interestingly, the toxicity of rutin alone increased with protein levels (see also Bloem and Duffey 1990). Among mammals, it is know that the saliva of browsing deer is richer in proteins and has a greater tannin-binding capacity than that of grazing sheep (Robbins et al. 1987, Austin et al. 1989). The natural food of deer is tannin-rich, and it has been suggested that these additional proteins reduce the toxicity of tannins for browsers (Austin et al. 1989). Such salivary proteins are expendable, since they pass out in the faeces of mammals as tannin-protein complexes. The production of these proteins may enhance the protein requirements of browsers, and these requirements may least be met when their foodplants simultaneously contain low levels of protein and high levels of phenolic compounds.

Dietary imbalance: restriction and augmentation of intake

Dietary imbalance is a concept central to ecological, evolutionary and physiological consideration of nutrition, and it is therefore unfortunate that this topic has received

so little explicit discussion in the entomological literature (see House 1966b, 1969, and Simpson and Simpson 1990 for exceptions). Dietary imbalance is a relative concept, and can be defined as a mismatch between 1) the nutritional requirements of an animal (which change in time) and 2) the availability of nutrients in a form in which they are commensurate with the genetic fitness of the animal. By the latter is meant that in a balanced diet, the required nutrients must be available at levels which will have a maximal contribution to an animal's fitness, given the combination of all other factors impinging on the animal at a given time. Considering an individual nutrient, this combination of factors includes the availability of other nutrients at non-limiting levels, without which the individual nutrient is by definition available in excess. Additionally, the presence of allelochemicals can restrict the utilization of an otherwise balanced complement of nutrients and may therefore also offset dietary balance. The same is true for indigestible bulk in foods so low in nutrients that compensatory feeding can at best be incomplete. All diets are to some extent imbalanced, but the effects of this may be averted by food selection, compensatory feeding and possibly adjustments in the efficiency with which insects utilize ingested compounds (Slansky and Scriber 1985, Waldbauer and Friedman 1988, Simpson and Simpson 1990). This definition of dietary

imbalance places emphasis both on individual dietary components and the ways in which these interact. For example, the interaction of tannic acid and protein in the present study can readily be interpreted as a case of tannic acid restricting compensatory feeding for proteins. Likewise, the interactive effects on consumption of protein and carbohydrate levels can be considered in this light. Here the situation becomes considerably more complex, however. This is because any consideration of interactions between two nutrients, either of which may cause compensatory feeding, must take into account a combination of four forces: upwards regulation for either nutrient, and the limitations imposed on this upwards regulation due to the mechanisms which signal repletion of the other nutrient. These forces may oppose or complement each other in various combinations. By contrast, tannic acid did not result in upwards regulation of intake, and may therefore only oppose the upwards regulation for low levels of proteins. It is as a means to describe these relationships in the regulation of intake that I introduce the terms 'incidental restriction' and 'incidental augmentation' of intake (see Results; Fig. 2).

The results of this study, as presented in Fig. 1, illustrate how two nutrients can interact in determining levels of consumption. Protein clearly influenced carbohydrate intake more strongly than vice-versa, and the

major mechanism accounting for this was incidental augmentation of carbohydrate intake, resulting from compensatory feeding for proteins. Nonetheless, high levels of proteins did limit intake of carbohydrates, but to a lesser extent than low levels of protein enhanced carbohydrate intake. The effects of carbohydrate levels on protein consumption were less marked, but nonetheless significant. In this case there was no apparent asymmetry between the roles of incidental restriction and augmentation of intake, with both mechanisms affecting protein intake to a similar extent.

It is therefore clear that incidental augmentation and restriction of intake are concepts of pertinence to the understanding of how insects compensate for nutrient deficiencies, and how dietary imbalance may interfere with this process. Simpson and Simpson (1990) addressed an aspect of this issue in their consideration of the possible consequences of behavioral compensation for low levels of dietary nutrients. They correctly point out that

" .. a completely overlooked consequence of compensatory feeding ... is the possibility that the control mechanisms regulating intake of different nutrient groups interfere with each other".

This they contrast with the common interpretation that

"..the poor performance of insects fed a nutritionally imbalanced diet is the result of their respiring or excreting nutrients which are excess to their requirements in order to gain sufficient of those which are limiting".

Consideration of the interplay of incidental restriction and

augmentation of intake reveals how these consequences of compensatory feeding may in many instances be simultaneous and causally related phenomena. For example, where the balance between two nutrients is skewed relative to the insect's requirements, upwards regulation for nutrient "b" might be limited by an inability to ingest more of nutrient "a". In this case, the insect might suffer a simultaneous deficit of nutrient "b" due to incidentally restricted intake, and a surplus of nutrient "a" due to incidentally augmented intake (e.g., see case 4 in Fig. 2). In the present study, an example of this may be found in the insects fed the P14-C28% diets. Table 2 shows that insects fed the 14% carbohydrate diets and those fed the 28% protein diets grew significantly less than those fed the 14% protein and 28% carbohydrate diets, respectively. Additionally, the means for the protein x carbohydrate interaction for total growth (Table 3) show that the lowest growth was achieved by the insects fed the C14-P28% diets (136.8 mg), although this interaction was not statistically significant. From Fig. 1 it can be seen that these insects experienced both incidentally augmented intake of proteins (point (i)) and restricted intake of carbohydrates (point (t)). Here restricted intake of carbohydrates may have limited the deposition of energy reserves, as may be the case for *Spodoptera eridania* (Karowe and Martin 1989) and *Argyrotaenia velutinana* (Lii et al. 1975), in which larval

fat content has been found to decrease with an increase in dietary proteins (see also Gordon 1972). Additionally, it is possible that there was a metabolic cost due to the ingestion of high levels of nitrogen (Scriber and Slansky 1981). Schroeder (1986) concluded that this was the case for *Datana ministra* which experienced reduced pupal weight when the leaves of the larval foodplant were coated with a protein supplement. In mammals, the activities of many enzymes required for the catabolism of amino acids increase when diets containing excess protein are ingested (Harper 1964 and refs therein).

A lack of clear focus on the basis of interactions between ingested compounds has in some cases lead to confusion in the design and interpretation of experiments. A common example of this has been the failure to distinguish direct physiological effects of dietary imbalance from those that may be primarily effects on consumption and only secondarily physiological; Grabstein and Scriber (1982) have made a similar point about the interpretation of experiments in which utilization efficiencies change when insects are switched from familiar to novel diets. Sang (1959, 1962), for example, found that axenically cultured *Drosophila melanogaster* larvae fed artificial diets containing 7% protein required higher dietary levels of vitamins to obtain the same rate of development as those fed 3% protein. This he interpreted to indicate that the increase of ingested

amino acids resulted in an increase in protein metabolism and a concomitant increase in vitamin requirements. While this may well be true for certain vitamins and amino acid levels, Sang did not measure levels of consumption and thus ignored an important factor. If intake were regulated according to protein levels, then incidental restriction of vitamin intake would result when protein levels were high. Therefore, contrary to the intentions of the experimental design, levels of vitamin intake - and hence, effectively, dietary levels - may actually have been higher when protein levels were low than for the same vitamin levels occurring in high-protein diets. Likewise, House (1965) found that *Celerio euphorbiae* increased consumption when the entire level of nutrients in artificial diets was diluted by the addition of water. When the balance of nutrients was altered by decreasing vitamin levels and increasing protein and amino acid levels, the larvae showed reduced consumption and growth. House suggested that these results may be due to the "metabolic difficulty" of eating an unbalanced diet. However, as has been pointed out by Simpson and Simpson (1990), the reduced consumption could reflect a regulatory response to increased protein levels rather than some direct, physiological deleterious effects of ingesting the imbalanced diets. Reduced growth would then result from the incidental restriction of intake of all other dietary components, including vitamins and digestible carbohydrates.

Similarly, in a study of the fly *Agria affinis*, House (1966a) varied independently the levels in artificial diets of amino acids, salts and other nutrients. When the levels of salts were kept constant, larval growth decreased with increasing levels of amino acid mixture but increased as the levels of other nutrients were increased simultaneously. Again, a regulatory response to increased protein levels may have resulted in the incidental restriction of intake of salts and other nutrients when protein levels were high. More recently, Schroeder (1986), in a test of the hypothesis that arbivorous insects are protein limited, found that *Datana ministra* experienced reduced net growth efficiency (ECD) when leaves of the larval foodplants were coated with a protein supplement. Schroeder suggested that this may be due an accumulation of excess nitrogenous metabolites. However, the protein supplements also resulted in reduced levels of consumption relative to controls, and it is therefore possible that reduced efficiency of conversion was due to reduced intake of some other dietary component(s). Redak and Cates (1984) found it "rather surprising" that female spruce budworm had reduced growth on Douglas-fir trees with higher nitrogen content, and suggested this may be a result of higher nitrogen being indicative of the trees' vigour and capability to resist insect attack. Alternatively, it might be that high nitrogen levels restricted intake of some limiting nutrient(s).

According to the definition of dietary imbalance given above, a balanced diet should be defined according to an independent criterion, the MOST IMPORTANT of which is contribution to fitness. Growth is a convenient and commonly used index of fitness (Slansky and Scriber 1985, Slansky and Wheeler 1990), but the underlying assumptions must be regarded with caution. In the present study, for example, insects fed low protein diets attained greater levels of total growth than those fed high protein diets. Nitrogen growth, on the other hand, was significantly greater on the diets containing high protein levels. In this case, if total growth were taken as the index of fitness it would have to be concluded that insects with higher body nitrogen content were less fit than the others, and this is clearly a moot point (see also Gordon 1972). The interesting possibility exists that there is an insect equivalent of obesity, and incidentally augmented intake of high levels of carbohydrates in order to compensate for low dietary proteins resulted in dry weight increment which correlates negatively with fitness. It could also be that a high level of dietary proteins limits fat deposition below the optimum for particular species (Slobodkin and Richman 1961, Calow and Jennings 1977) through the incidental restriction of lipid and carbohydrate intake, and thereby reduces fitness. A negative relationship between dietary protein levels and fat deposition has been observed in several species, including

insects (Hirano 1964, Gordon 1972, Lii et al. 1975, Karowe and Martin 1989), chicks (Donaldson et al. 1956, Velu et al. 1971), ducks (Scott et al. 1959) and turkeys (Donaldson et al. 1958). Slansky and Wheeler (1989, 1990) found the opposite to be true for the velvetbean caterpillar, *Anticarsia gemmatalis*. In this case total dietary nutrients were diluted with cellulose or water, so that the relative proportions of proteins, carbohydrates and lipids remained constant, and a negative relationship would not be expected.

Post-ingestive effects

The utilization efficiency of nitrogen by herbivorous insects has been found in numerous studies to be inversely correlated with dietary protein levels (e.g., Ito and Mukaiyama 1964, Nation and Thomas 1965, Bhattacharya and Waldbauer 1972, Mullins and Cochran 1975, Slansky and Feeny 1977, Scriber and Slansky 1981, Ohmart et al. 1985, Slansky and Wheeler 1989, Karowe and Martin 1989, Jindra and Sehnal 1989). While the same was true in the present study, the pattern was in this case not simple. Less nitrogen was ingested by insects fed the C28% than the C14% diets, but an increase in the efficiency at which nitrogen was converted for growth (ECI(N)) (i.e., a decrease in the proportion of ingested nitrogen voided with the faeces) resulted in a constant level of nitrogen accumulation (NG). By contrast, the insects fed the P14% diets also had lower

nitrogen consumption than those fed the P28% diets, but in this case there was no increase in ECI(N) and nitrogen accumulation was therefore reduced (Table 2). The interaction between carbohydrates and proteins (Table 3) suggested, however, that ECI(N) was particularly high for insects fed P14-C28% diets, the same diets on which the least protein and the most carbohydrates were consumed (Fig 1). It therefore seems that increased ECI(N) with low levels of nitrogen was dependant on the simultaneous ingestion of high levels of carbohydrate. Low carbohydrate levels may result in a larger proportion of amino acids being deaminated for use in energy metabolism (e.g. Moloo 1977), and hence the increased levels of nitrogen in the frass. There is also evidence for birds and mammals that the efficiency of nitrogen utilization may depend on the dietary calorific content (Yoshida et al. 1957 and refs therein).

While it remains an interesting possibility (Slansky and Scriber 1985, Simpson and Simpson 1990), increased utilization of diets containing low levels of specific nutrients does not necessarily indicate that the insects compensated post-ingestively for those nutrients. There are several potential problems with this idea. First among these is the question of causal relationships (Scriber and slansky 1981, Simpson and Simpson 1990): it is difficult to determine whether decreased intake resulted in increased utilization or vice-versa. Secondly, any true compensatory

response is the expression of actively regulated control mechanisms (see Calow 1976, Slansky 1982), which should be modulated by feedback information about the animals' nutritional state (Simpson and Simpson 1990). Without discovering the actual mechanisms, it is difficult to obtain direct evidence that regulation is active, although where nutrients are regulated individually this may provide circumstantial evidence (Simpson and Simpson 1990).

A third problem is the question of mechanisms. Regarding digestion for example, there is evidence for several species of herbivorous insects, including *L. migratoria* (Clarke and Gillot 1967, Chapman 1985b), *Heliothis zea* and *Spodoptera exigua* (Broadway and Duffey 1986), that the secretion of proteolytic enzymes is under the control of a secretagogue mechanism. As Simpson and Simpson (1990) have pointed out, it is problematic to explain how a secretagogue mechanism could enable larvae feeding on food low in proteins to increase nitrogen utilization efficiency; it is more likely that digestive efficiencies would actually decrease with decreasing levels of dietary protein.

An additional, theoretical, point is the question why animals capable of utilizing, for example, nitrogenous nutrients with high efficiency should do so only under conditions of nutritional stress. Available nitrogen is often a limiting factor to herbivorous insects (Mc Neill and Southwood 1978, Mattson 1980, Scriber and Slansky 1981), and

insects that can obtain this more efficiently from their environment will have obvious fitness benefits. One such benefit may be a reduction in foraging time, and this may have energetic advantages (Aidley 1976, Calow 1977, McEvoy 1984, Slansky and Wheeler 1990) and may decrease the risk due to predators and parasitoids (Heinrich 1979, Slansky and Feeny 1977, Slansky and Scriber 1985). Additionally, since increased efficiency of utilization may decrease the amount of food eaten, the incidental ingestion of harmful allelochemicals, pathogens etc. may be reduced.

Given these problems, it may be heuristic to approach the issue from a different angle, considering increased *excretion* of specific nutrients when *high levels* are ingested rather than increased utilization of low levels. This is concordant with a secretagogue mechanism for control of digestion, since there must be a limit to increased enzyme secretion, beyond which any further intake of a nutrient would result in its undigested egestion. Additionally, this approach provides a basis for considering correlations between levels of intake and utilization efficiencies in the light of interactions between nutrient groups and between pre- and post-ingestive phenomena. For example, where nitrogenous nutrients are present in a diet in excess relative to some other nutrient for which there is compensatory feeding, incidental augmentation of intake will result in the ingestion of high levels of nitrogen (as

depicted in Figs 1 and 2). A point may be reached at which the effects of nitrogen accumulation stop further consumption, resulting in the incidental restriction of intake of any limiting nutrients. The extent of this restricted intake will be inversely proportional to the ability of the insects to egest or excrete selectively the excess of nitrogen (and hence proportional to AD and ECI for nitrogen). From this viewpoint, correlations between the efficiency of utilization of nutrients and their intake may suggest post-ingestive mechanisms facilitating compensatory feeding for *other* nutrient groups. A further, interesting possibility is that the mechanisms regulating intake of macro nutrients (e.g., carbohydrates, proteins) have been set by natural selection at levels which ensure the intake of more of these nutrients than the digestive physiology can process. This would increase the chances of obtaining enough of other, perhaps micronutrients, for which there are not specific regulatory mechanisms. When the macronutrients are in short supply in a diet, the excess egested with the frass will be reduced (with no actual change in the digestive physiology) giving the impression that utilization efficiency increased.

The foregoing does not exclude the possibility that there may be true post-ingestive compensation (as opposed to post-ingestive phenomena facilitating compensatory feeding), but provides an additional possible explanation for the commonly

observed correlations between ingestion of specific nutrients and the efficiencies with which they are utilized. While pertinent studies of interactions between major nutrient groups are virtually non-existent, published studies on the effects of amino acid imbalances in insects may provide information for distinguishing these two possibilities. Several authors have found that nitrogen excretion or egestion increases in insects fed diets containing poor quality proteins or a measured imbalance of amino acids (Horie and Inokuchi 1978, Scriber and Slansky 1981, Horie and Watanabe 1983, Karowe and Martin 1989), and the same has been found for mammals (Albanese 1959). It may be that such increased nitrogen excretion facilitates further intake of those amino acids which are limiting. This would rely on a degree of selective egestion, excretion or catabolism of specific amino acids ingested in excess (Harper 1964). The amino acids arginine and histidine are excreted selectively by females of the blood-feeding *Glossina morsitans* (Moloo 1978), and this selective excretion appears to be modulated by the insects' nutritional state since it slows during pregnancy when these amino acids are utilized in synthetic processes associated with larval nutrition (Moloo 1977). Alternatively, if it were found that there was, selectively, increased absorption and decreased excretion or catabolism of the limiting amino acids, this would suggest true post-ingestive compensation.

Selective absorption of amino acids has been demonstrated in larvae of *Bombyx mori* (Shyamala and Bhat 1966), but it is not known whether this selectivity is altered according to the nutritional status of the insect and would thus represent a true compensatory mechanism. Perhaps the most convincing evidence to date for active regulation is that of Van Loon (1988), who demonstrated that *Pieris brassicae* and *P. rapae* are able to regulate utilization efficiencies of individual amino acids depending on the levels consumed. However, without elucidating the underlying mechanisms it is not possible to determine whether increased utilization of limiting amino acids, decreased utilization of those ingested in excess or both occurred.

Conclusions

The use of artificial diets in nutritional research has enabled researchers to eliminate unwanted covariates, while assessing the effects of individual compounds on insect nutrition (Dadd 1960b). Plant compounds do not, however, occur in isolation, and there is now considerable evidence to suggest that their effects on herbivores can be interactive. These interactions should not be regarded as merely of practical interest to researchers wishing to avoid the confounding effects of uncontrolled variables; they should be recognized as an important subject of study in their own right. The simultaneous variation of two or more

compounds in factorial experiments using artificial diets provides a powerful tool for investigating such interactive effects of plant chemicals on insects. In this way, the unidimensional approach currently dominating research in the field of plant/herbivore interactions (Duffey et al. 1986) may be substituted BY a more realistic one which takes into account the complex network of causation characteristic of biological systems (Caswell et al. 1971). The results of the present research have illustrated some ways in which interactions between coingested compounds can be an important consideration in insect nutritional studies. It is hoped that this developing aspect to the study of insect nutrition will be incorporated into ecological theory, providing a more holistic basis for the understanding of how plants and insects interact in ecological and evolutionary time.

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Chapter 3

Dietary Mixing in Grasshoppers: Changes in Acceptability of Different Plant Secondary Compounds Associated with Low Levels of Dietary Protein

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Abstract. *Schistocerca americana* sixth instar nymphs were examined for a change in diet acceptance, in which insects experiencing an unfavorable diet subsequently become predisposed to eat relatively less of that diet and more of diets with a novel flavor than they would had they previously fed on a more adequate diet. Insects were pretreated for 4h on either low (2% wet wt.) or higher-protein (4%) artificial diets flavored with a plant secondary compound (tomatine or rutin). They were then offered in choice or no-choice tests the low-protein diet with the familiar or a novel (tomatine, rutin or NHT) flavor. When tomatine was the familiar and rutin the novel flavor in a no-choice test, the insects previously fed low-protein diets took relatively long meals on the novel and relatively short meals on the familiar diets compared with the insects that had previously eaten higher-protein diets. A similar, but in this case considerably less pronounced and statistically non-significant, pattern existed in the reciprocal design experiment in which rutin was the familiar and tomatine the novel flavor. Similarly, insects fed low-protein diets flavored with rutin subsequently showed increased relative preference for the novel flavor (NHT) in a choice test, compared with the high protein-pretreated insects. It is concluded that insects fed protein-deficient diets may subsequently show a preference for novel foods through different mechanisms, the importance of which may differ under different circumstances.

INTRODUCTION

Polyphagous grasshoppers commonly show a faster growth rate and increased reproductive output when they consume a mixture of plant foods than when reared on one alone. One of the hypotheses presented in explanation of this is the need to select a variety of foods to ensure an adequate nutrient balance, since plants vary considerably in both major and minor nutrient levels.

The mechanisms involved in dietary switching are, however, not clear and various possibilities could explain the observed abilities. For example, changes in sensitivity of chemoreceptors and an ability to monitor post-ingestively the nutrient levels of foods has been demonstrated (Abisgold and Simpson, 1987; 1988), and recently it has been demonstrated that different protein types can actually be detected by smell (Heinrichs et al. 1990). On the other hand, Waldbauer and his coworkers suggest a malaise induced by imbalance of nutrients, leading to movement away from the food being eaten and subsequent encounter with a new food item (Cohen et al., 1987; Waldbauer and Friedman, 1988). Additionally, learning is probably important in insects as it is in vertebrates: Simpson and White (1990) demonstrated that grasshoppers can learn to associate high protein levels with odors, so that over time positive associations may be an important part of dietary mixing; Lee and Bernays (1988;

1990) and Champagne and Bernays (in press) have demonstrated that grasshoppers are capable of learning to avoid foods with noxious associations including post-ingestive poisoning or a lack of utilizable sterols. It has, further, been proposed that dietary mixing in rats (Rozin, 1976) and cockroaches (Geissler and Rollo, 1988) may be facilitated by a "novelty effect", in which experience with an unsatisfactory diet predisposes the animal to subsequently eat more of novel foods. A novelty effect could be due to a true neophilic response, or a secondary consequence of aversion learning in which the animal eats more of a novel diet simply because it eats relatively less of the familiar diet with negative associations.

This study investigates changes in the acceptability to an herbivorous insect of flavors which had previously been associated with high or low-protein diets, and attempts to elucidate the relative roles of aversion learning and neophilia in this response. I used the polyphagous grasshopper, *Schistocerca americana*, and artificial diets flavored with plant secondary compounds.

METHODS

Culture

Insects used were *Schistocerca americana* (Drury) nymphs, that had been kept in culture for many generations. They

were kept in similar conditions to those recommended by Hunter-Jones (1961) and fed on seedling wheat, lettuce and wheat bran. For tests, insects were of standard age. Newly molted sixth instar nymphs were sorted from the stock cage daily; groups of 12 to 40 individuals were placed in plastic cages of eight liter capacity and held in a controlled environment room at a mean temperature of 30°C and a cycle of light:dark 12:12h. They were fed fresh seedling wheat daily. All behavioral tests were carried out at 30°C.

Establishing the experimental design

It has been shown in the locust, *Locusta migratoria*, that after eating a diet low in either carbohydrate or protein, individuals will subsequently show a preference for the complementary diet when given a choice between the two (Simpson et al., 1990). For protein, an effect can be monitored after a single meal, and is maximal after 4h on the unbalanced diet. On the basis of this and of preliminary observations, I chose 4h on the pretreatment diets as a time when several meals had been taken, but a minimum of growth effects due to any differences in protein level could have occurred. Individuals were allowed to feed for 4h with no choice on diet with one of two protein levels, before being tested in choice or no-choice situations for first meal lengths on new foods.

The selection of two protein levels was also based on

work in other species. It has been shown in both *L. migratoria* and *Schistocerca gregaria* that more feeding occurs on a diet low in protein than on a diet high in protein (Chapters 1 and 2, Simpson and Simpson, 1990). On dry diets it has been found that at concentrations of 7% dry weight protein, individuals fed maximally but had lower body nitrogen than those which had received higher levels of protein (Raubenheimer and Simpson, in prep.). On this basis, 14% dry weight was selected as the high concentration in the present study, and 7% as the low concentration (henceforth referred to as the P14% and P7% diets). These diets were further diluted by water in the preparation of an agar-based diet cake (see below) to approximately 4% and 2% on a wet weight basis. This would mean that all individuals would be expected to be feeding maximally, ensuring that the time spent feeding was similar in the high and low-protein treatments and that all nutrients except protein were ingested in similar quantity. Preliminary tests of this for the new species and with the different diet formulation established that the individuals did indeed feed in similar manner on the two different diets over the 4-h pretreatment period. Using diets flavored with rutin, total times spent feeding (mean $\pm$ s.e.) were 32.2 $\pm$ 3.2 minutes (n=20) on the P7% and 29.8 $\pm$ 3.5 minutes (n=16) on the P14% diets ($p>0.33$, one-tailed t-test). Average meal durations were 6.1 $\pm$ 0.73 and 7.2 $\pm$ 0.81 ($p>0.28$) for the P7% and P14% insects,

respectively.

The selection of plant secondary compounds with which to flavor the food was based on information from two-choice experiments on *S. gregaria*, using as a substrate filter paper discs (Bernays, unpublished). It was considered that the ideal tastes would be compounds that were phagostimulatory at low concentration and deterrent at high concentrations, so that they could be added at levels that might be in between. This would provide compounds that did not directly influence food choice but should be tasted. In practice, it was difficult to balance the direct effects of the three tastes. Results were somewhat different with *S. americana* and apparently very different with the artificial diets compared with choice tests carried out with filter paper discs.

For these pilot experiments, individuals were continuously monitored in plastic boxes with one or the other diet, using a laptop computer programmed as an event recorder. First meal lengths were used as a measure of relative acceptability. Observations were made on ten separate individuals at any one time.

The differences in the effects of the compounds used were consistent as to direction but considerable differences occurred from day to day in first meal lengths on all four diets. Overall preference ranking however, was as follows: rutin diet (0.4% dw) > tomatine diet (1.5% dw) > nicotine

hydrogen tartrate - NHT (0.1% dw) > plain diet, at a ratio of 3 : 2 : 1.25 : 1.

The experimental design

In all cases first meal duration was used as a measure of palatability, since this precluded any effects of experience resulting from more prolonged contact with the diets. All experiments were carried out in two balanced replicates.

A. Pretreatments followed by no-choice tests

Experiment 1

Pretreatment: insects were given either P14% + tomatine or P7% + tomatine for 4h.

Test: in each case half were tested with P7% + tomatine and half with P7% + rutin, and first meal length was recorded.

If aversion learning of the diet with extremely low protein were involved, it would be expected that those insects pretreated on P7% + tomatine would tend to eat a relatively small meal of P7% + tomatine in the test. If neophilia occurred, insects pretreated on P7% + tomatine would be expected to eat relatively longer meals of P7% + rutin than those that had had P14% with tomatine in the pretreatment. Since these two processes probably occur together however, they cannot be separately identified with this experimental design.

Experiment 2

This was the reciprocal design of Experiment 1, using rutin as the flavor in the pretreatment and tomatine or rutin in the test.

B. Pretreatment followed by choice tests

Experiment 3

This experiment involved a similar pretreatment to Experiment 2, using rutin as the added chemical. However, in the test period individual insects were allowed a choice between P7% + rutin and P7% + NHT. Reasons for employing a choice test were firstly, to examine which diet was selected first, and secondly the test may be more sensitive than a no-choice test. This is because individuals are given the opportunity to switch diets, which allows both positive and negative stimuli to operate in the same test (Cook, 1976).

Experimental Regime

The diet used was based on that used by Simpson and Abisgold (1985) with 14% dry weight of protein (cf. the P14% diets in Chapters 1 and 2). Further dilution to 7% protein was achieved by replacing 7% of the protein with (indigestible) cellulose, thus ensuring that protein was the only nutrient compound to change in concentration. However, instead of using dry diet and a separate source of water, the diets were incorporated into small cakes using 1% agar. Eight

grams of diet was mixed with 24ml of the agar solution at 40°C. The mixture was poured into plastic molds 3cm in diameter and 5mm deep. After setting, the small cakes could be taken out of the molds and used directly in experiments. These cakes have several advantages over dry diet for short-term behavioral experiments. First, the edges provide an appropriate physical stimulus for feeding on first contact. Second, there is no need for insects to learn how to handle very small particles. Also, because of the presence of water in the diet, complete meals can be taken without interruption for drinking. Protein levels at the start of experiments were approximately 4% and 2% on a wet weight basis.

Insects used were 3-4 days old in the 11-day instar. They were placed in the experimental boxes and deprived of food for approximately 18h prior to feeding in the pretreatment. This ensured that all individuals began feeding early in the pretreatment period. Pretreatments usually began at about 08.00 and tests at 12.00 h. All pretreatments and observations were made in an observation chamber held at 30°C. The insects were contained in plastic boxes measuring 21.5cm high x 11.0cm wide x 3.5cm deep with gauze-covered ventilation holes measuring 25mm in diameter at each end. Boxes were screened from one another to prevent any mutual disturbance of the insects. In the no-choice tests, diet was placed centrally in the bottom of the box. In the choice

experiments, diets were placed symmetrically on the bottom about 4cm apart. Usually ten individuals were observed by one person at any one time. All recording of feeding behavior used a laptop computer programmed as an event recorder. Records were made as follows: Palpation, Biting, Feeding, Leaving food.

Definitions of feeding behavior were:

Meal: sum of feeding periods with the insect usually staying in contact with the diet. The end of a meal was signified by not feeding for five minutes, during which the insects almost always left the diet and roosted on the walls of the container.

Rejection: biting the food but leaving rather than staying to feed. In choice tests, individuals sometimes moved directly from one diet to the other. Leaving the first diet signalled the end of a meal on it. In a few cases during choice tests individuals embedded a meal of one diet in a meal of the other. In this case, additional feeding on the first diet was included in the meal on that diet if it occurred within five minutes of leaving the second diet. Data used included only individuals feeding on both diets within two meals.

RESULTS

No-choice Experiments

The data for first meal durations in both no-choice experiments were found to be normally distributed ($P>0.3$; Kolmogorov-Smirnov test) and with similar variance ($P>0.1$; Bartlett's test). ANOVA was therefore used, with pretreatment (7% or 14% protein) and flavor (novel or conditioned) as factors with two levels each. A significant two-way interaction between pretreatment and test diet flavor would indicate that the relative response to novel and familiar diets was influenced by the protein levels in the pretreatment diets. In addition, since the data for each experiment were collected in two balanced replicates, replicate was included as a two-leveled factor in the models. A significant main effect of replicate would indicate that the means differed between the two replicates but the pattern of the data was similar, while any significant interactions involving replicate would suggest a qualitatively different outcome on different days. There were, however, no significant main effects or interactions involving replicate (Tables Ia and b), and the means presented (Tables IIa and b) therefore represent the combined data for both replicates of each experiment.

a) *Experiment 1: tomatine pretreatment*

Table Ia shows that there was a significant flavor x

pretreatment interaction for the duration of the first meal in the test period. Inspection of the means and standard errors presented in Table IIa reveals that meal duration on the novel diets was relatively long and that on the familiar diets relatively short for the insects pretreated on P7% diets compared to those previously fed the P14% diets. This demonstrates that the relative acceptability of novel and familiar flavored diets changed depending on the levels of protein in the diets on which the insects had previously fed.

b) *Experiment 2: rutin pretreatment*

When insects had been preconditioned on rutin-flavored diets, there was no significant flavor x pretreatment interaction in the duration of the first test meal (Table Ib). Notwithstanding this lack of statistical significance, the pattern of means followed a similar trend to Experiment 1, in which the ratio of meal size of novel/familiar diets was greater for the P7%- than the P14%-pretreated insects. There was a significant main effect of pretreatment and Table IIb shows that the insects pretreated on P7% diets took longer meals than those previously fed P14% diets. Additionally, there was a significant main effect of diet flavor (Table Ib), based on the fact that longer meals were taken on the rutin than the tomatine diets (Table IIb).

Table I. ANOVA tables for durations of first meal of *S. americana* nymphs pretreated on diets containing 7 or 14% protein flavored with tomatine (a) or rutin (b) then tested on 7% protein diets containing the familiar or, reciprocally, a novel flavour.

(a) Tomatine pretreatment

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN CELLS	846.34	31	27.30		
PRETREATMENT	0.80	1	0.80	0.03	0.865
FLAVOR	128.35	1	128.35	4.70	0.038
REPLICATE	73.82	1	73.82	2.70	0.110
PRET BY FLAVOR	126.50	1	126.50	4.63	0.039
PRET BY REP	3.91	1	3.91	0.14	0.708
FLAVOR BY REP	15.22	1	15.22	0.56	0.461
PRET BY FLAVOR BY REP	0.62	1	0.62	0.02	0.881

(b) Rutin pretreatment

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN CELLS	161.99	32	5.06		
PRETREATMENT	38.14	1	38.14	7.53	0.010
FLAVOR	171.23	1	171.23	33.83	0.000
REPLICATE	18.60	1	18.60	3.68	0.064
PRET BY FLAVOR	0.78	1	0.78	0.15	0.698
PRET BY REP	3.31	1	3.31	0.65	0.425
FLAVOR BY REP	12.37	1	12.37	2.44	0.128
PRET BY FLAVOR BY REP	0.10	1	0.10	0.02	0.888

(MINS ± S.E.)

Table II. First meal durations for *S. americana* nymphs fed 7 or 14% protein diets combined with tomatine (a) or rutin (b) then offered 7% protein diets containing either the familiar or, reciprocally, a novel flavor.

pretreatment diet	Test flavor: familiar (tomatine)	Test flavor: novel (rutin)
(a)		
7% pro+tomatine	3.07±1.16 (n=10)	10.32±2.24 (n=10)
14% pro+tomatine	6.40±1.44 (n=10)	6.39±1.59 (n=9)

	familiar (rutin)	novel (tomatine)
(b)		
7% pro+rutin	7.5±1.06 (n=10)	3.6±0.60 (n=10)
14% pro+rutin	5.8±0.69 (n=10)	1.4±0.38 (n=10)

Choice experiment

(*Experiment 3*)

Table III shows the first meal durations on both the conditioned (rutin) and novel (NHT) flavored diets. As in the no-choice experiments, the ratio of mean first meal duration on novel/familiar diets was greater for the P7%- than the P14%-pretreated insects. Insects preconditioned on P14% diets flavored with rutin took significantly longer first meals during the test period on the familiar than the novel diets. This could be expected considering the relatively strong phagostimulatory nature of rutin. There was, however, no such statistically significant difference for insects which had been fed low-protein diets in the pretreatment. This difference in the outcomes for P14% and P7%-pretreated insects suggests that, as in the no-choice experiments, the relative attractiveness of novel and familiar flavors was influenced by the protein levels of diet on which the insects had previously fed.

Insects in both pretreatment groups tended to take their first meal during the experimental period on the novel-flavored diets, although in neither case was there statistical significance. Of the insects pretreated with P7% diets, 5 took their first meal on the rutin diets and 10 on the NHT diets (P=0.3; binomial distribution) and of the P14% insects, 5 fed first on rutin flavored diets and 9 on NHT diets (P=0.42). The combined 2-tailed probability for both groups is 0.14.

Table III. First meal durations (mean ± s.e. mins.) of sixth instar *Schistocerca americana* pretreated for 4h on rutin-flavored diets containing 7 or 14% protein then given a choice of 7% protein diets containing the familiar or a novel (NHT) flavor. P-values are for a *t*-test of significance between preference index means and zero, the expected value if there was no difference between first meal durations on the two test diets.

	Test flavor				
Pretreatment	Familiar (rutin)	Novel (NHT)	Preference index (rutin-NHT)	t-value*	Sign. of t
7% pro (n=15)	3.69±0.80	2.49±0.76	1.19±0.95	1.26	0.23
14% pro (n=14)	4.06±1.24	1.05±0.25	3.01±1.15	2.61	0.02*

* a parametric test was justified here because the preference index for both groups were found to be normally distributed (P=0.37 and P=0.34 for the P7%- and P14%-pretreated groups, respectively).

DISCUSSION

The present data have demonstrated that the relative acceptability of novel and familiar flavors may change depending on the levels of protein in the diets with which the familiar flavor had previously been paired. Of three experiments performed, there was in all cases a tendency for the insects pretreated with low protein diets to take relatively larger first meals on the novel than the familiar diets compared to the insects that had previously been fed diets containing higher levels of protein. This was, however, a complex phenomenon and there were marked differences in the strength and details of this effect depending on the relative phagostimulatory ability of the conditioned and familiar diets, and whether the effects were measured in choice or no-choice assays.

Where the more phagostimulatory rutin was the novel flavor in a no-choice test (Experiment 1), there was a relatively strong, statistically significant effect in which the ratio of mean first meal size on the novel diet/familiar diet was larger for the p7%-pretreated than the p14%-pretreated insects. A comparable, but in this case statistically non-significant pattern, was obtained for the reciprocal experiment (Experiment 2), in which rutin was the familiar and tomatine the novel flavor. Similarly, in the choice experiment, using rutin as a familiar and NHT as a novel flavor (Experiment 3), the insects that had previously fed on P14% diets showed a strong preference for

the more phagostimulatory rutin diets, while the preference was considerably less marked for insects pretreated on low-protein diets. This again suggests that the novel-flavored diets became relatively more acceptable than familiar ones for insects that had previously been fed lower- than those fed higher-protein diets.

These results are thus consistent with an induced novelty effect in which an insect experiencing a nutritionally or otherwise unfavourable diet subsequently becomes predisposed to eat relatively more of diets with a novel flavor than it would had it previously fed on a more adequate diet, thus facilitating dietary mixing. An induced novelty effect could come about through one or a combination of several mechanisms, which are experimentally difficult to tease apart.

For example, the pattern of means obtained for the no-choice experiment in which tomatine was the pretreatment flavor (Experiment 1) may be explained by a number of possibilities. There was, compared to the P14%-pretreated insects, a relatively long first meal on novel diets and a relatively short one on familiar diets by the insects previously fed low-protein diets. This could involve decreased attractiveness of the familiar-flavored diet. If so, this would indicate aversion learning, in which protein deprivation would serve as the unconditioned stimulus which becomes associated with the conditioned stimulus, the flavor of tomatine. In addition, or alternatively, the long meal

on the novel-flavored diet could indicate that there may be a specific neophilia, in which insects respond preferentially towards novel flavors, as opposed to ingesting relatively more of a novel diet as a secondary consequence of rejecting the familiar ones. There is, however, an additional complication in interpreting this pattern. Since the P7% insects had ingested less protein during the pretreatment than the P14% insects, it may be that the increase in first meal size was partly a result of compensatory feeding. That this might be the case is suggested by the significant main effect of pretreatment in Experiment 2. However, if compensatory feeding were the only explanation, it would be expected that first meal duration of the P7%-pretreated insects would be elevated on both the novel and familiar diets relative to the P14%-pretreated insects. This was clearly not the case (Table IIa), suggesting that there was aversion learning and/or neophilia.

Aversion learning based on nutritionally inadequate diets has previously been demonstrated for *S. americana* (Champagne and Bernays, in press), in this case in relation to the absence of suitable dietary sterols. *S. americana* also shows aversion learning when a meal of otherwise acceptable food is followed by an injection of toxins (Bernays and Lee, 1988; Lee and Bernays, 1990).

The nature of the familiar and novel flavors may be an important factor in the processes of aversion learning and

neophilia, and the lack of a statistically significant pretreatment x flavor interaction in Experiment 2 is probably due to big differences in acceptability of the different tastes as measured on naive insects. Here rutin, a compound which is known to be a powerful phagostimulant for *S. americana* (Bernays et al. in press), was the pretreatment flavor. At the concentrations used, it is a more powerful phagostimulant than tomatine and this is further SUPPORTED by the significant main effect of flavor in the analysis of this experiment. In nature, phagostimulants usually signal nutritionally suitable foods and on this basis it might be expected that compounds with strong phagostimulatory effects would serve as relatively poor conditioned stimuli in aversion learning. There is, additionally, evidence for this since Lee and Bernays (1990) have found that aversion learning in *S. americana* did not occur with the most favored foods but only with foods that are less acceptable.

There was stronger evidence for a novelty effect when rutin was used as the pretreatment flavor in a choice rather than a no-choice experiment (Experiment 3). Choice tests are more sensitive than no-choice experiments (Cook, 1976; see also Chapter 1), since individuals may switch diets and this allows both positive and negative stimuli to operate in the same test. As in the no-choice tests, the difference in the outcomes for the P14% and P7% insects could result from either a relatively short time spent feeding by the P7%

insects on the familiar diets (conditioned aversion) or a relatively long time spent feeding on the novel diets (neophilia), or both. Inspection of the data presented in Table III suggests that in this case the major effect was through neophilia: the mean first meal duration of the P7%-pretreated insects on the familiar diet was 0.9 times the size of that taken by the P14%-pretreated insects while on the novel diet, the mean meal duration was greater for the P7%- than the P14%-pretreated insects by a factor of 2.4.

Therefore, while these data suggest a novelty effect in both choice and no-choice experiments, there is a suggestion that the relative importance of neophilia was greater in the former, and aversion learning greater in the latter tests. In Chapter 1 I found in detailed behavioral experiments that, over a 12-h period, *L. migratoria* showed no detectable response to 10% tannic acid in artificial diets while the same compound was deterrent in a choice assay. This provides another example illustrating that different dietary-selection mechanisms may be elicited in choice and no-choice situations.

It thus appears that the various mechanisms underlying dietary mixing may have different relative importance under different circumstances, depending on such factors as nutritional status of the individuals, previous experiences they have had and whether or not an alternative food is available.

A novelty effect has previously been demonstrated in cockroaches (Geissler and Rollo, 1988), but no attempt was made to deal with the relative importance of different associated mechanisms such as aversion learning and neophilia. This may be difficult on the basis of behavioral experiments alone but I wish to emphasise that a "novelty effect" does not necessarily imply the existence of a specific neophilic mechanism. Animals can become predisposed to eat more of novel foods through a number of mechanisms, and it is possible that these may interact in various combinations producing the same behavioral outcome: feedback-mediated dietary mixing. A similar situation has been found for rats, where there has been a considerable amount of work on the capacity to make "nutritionally wise" decisions, and several different processes have been indicated (McFarland, 1985, pp 334-356).

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Chapter 4

Feeding patterns and dietary mixing in the polyphagous grasshopper *Taeniopoda eques*: a field study

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Abstract. Detailed field observations were made of the feeding behaviour of the polyphagous grasshopper *Taeniopoda eques* (Burmeister) in its desert habitat. Thirteen adult females were observed continuously throughout the day, including 3 on overcast days and 10 on sunny days. An additional 29 insects were observed for shorter periods ranging from 20 to 404 minutes. Insects observed on sunny days had two foraging periods, one in the morning and one in the afternoon, separated by an extended period of roosting. By contrast, those observed on overcast days had no extended midday roost and tended to feed intermittently throughout the day. Analysis of the patterns of feeding showed that feeding events were clustered into meals separated by intermeal intervals of 8 minutes or longer. Insects almost always ascended a perch and took postprandial rests between meals. Those observed on sunny days locomoted less and spent a greater proportion of the intermeal intervals quiescent, partly as a result of taking longer postprandial rests. The time spent feeding within meals was positively related to the amount of time locomoting in the preceding intermeal interval, and negatively related to the time locomoting in the following intermeal interval. A high degree of polyphagy was observed, including feeds on at least 53 plant species from 16 families. Meals consisted of feeds on up to 11 different food items, and individual insects fed on up to 30 items per day. Analysis of the

patterns of host switching revealed a tendency for successive feeds of the same food item to decline and those on different items to increase in duration. Possible mechanisms underlying this switching behaviour are discussed.

INTRODUCTION

In recent years, two areas have been the focus of much research in the fields of insect nutrition and nutritional ecology: the complex interaction of intrinsic and extrinsic factors involved in the regulation of food intake (reviewed by Barton Browne 1975; Bernays & Simpson 1982; Simpson & Bernays 1983; Bernays 1985; Simpson & Simpson 1990) and the selection by insects of specific foods (or combinations) from among a range of possibilities (Scriber & Slansky 1981; Simpson & Simpson, 1990; Waldbauer & Friedman 1991).

Carefully controlled laboratory studies involving the detailed analysis of feeding behaviour, often in conjunction with chemically defined artificial diets, are playing an increasingly major role in both areas (Chapter 1; Simpson 1990; Simpson & Simpson 1990; Waldbauer & Friedman 1991). For example, the regulation of intake in locusts (Simpson 1990; Simpson & Simpson 1990), blowflies (Dethier 1976; Simpson et al. 1989) and caterpillars (Reynolds et al. 1986; Bowdan 1988) have all been studied in this way. Likewise,

recent progress in understanding the mechanisms involved in dietary mixing in grasshoppers has come from such studies. The processes invoked include: chemosensory changes in relation to specific nutrient deficits (Abisgold & Simpson 1987, 1988); associative learning of odours and nutrients (Simpson & White 1990); aversion learning of toxins (Lee & Bernays 1988, 1990) or nutrient deficiency (Champagne & Bernays in press); and possibly neophilia in conjunction with aversion learning (Chapter 3).

It is one thing, however, to elucidate a behavioural mechanism under highly controlled laboratory conditions but quite another to assess its role under the complex conditions of a natural habitat. There have to date been no attempts to record in detail the feeding behaviour of individual grasshoppers in the field. Indeed, even studies on the range of foods eaten by grasshoppers are generally at the population level (Chapman 1990), so that the extent to which individuals mix diets has in most cases not been monitored (an exception is the study of Ben Halima et al. 1985).

This paper is a description and analysis of the detailed feeding behaviour in the field of a polyphagous grasshopper, *Taeniopoda eques*, based on continuous observation of individuals over time periods of up to 690 minutes. Insects were observed both on days in which there was no cloud cover and when cloud cover predominated, providing an opportunity to assess the effect of weather conditions.

METHODS

Insects and study sites

Taeniopoda eques (Burmeister) is a large aposematic (Whitman et al. 1987) species in the subfamily Romaleinae. It is visually conspicuous, having bright yellow markings set against a black background, and has a relatively high threshold for disturbance by large animal movements. As a species, *T. eques* is known to be polyphagous (Whitman & Orsak 1985), and preliminary studies (D. Champagne, pers. com.) indicate that individuals sequester noxious plant metabolites to add to their defensive secretions as does the related *Romalea guttata* (Jones et al. 1989).

Its visual conspicuousness and high threshold for disturbance make this species an ideal insect herbivore for field observation. Furthermore, the insects forage only during the daylight hours, roosting in trees and shrubs above ground as the temperature drops following sunset. This enables recording of the full daily diet during the 12-h period between sunrise and sunset. Adult females were selected for study because initial observations indicated that they were easier to track than adult males, which are smaller and able to fly. Nymphs appeared to have a lower threshold for disturbance, and were less suitable because of non-feeding periods before and after ecdysis.

T. eques typically inhabits semidesert grassland

communities with a sparse overstory of small to medium-sized *Acacia* or *Prosopis* trees, at elevations between approximately 1000 and 1600 m. Insects hatch in about July after the first summer rains, so that during most of their lives they can feed on new plant growth including ephemeral herbs, seedlings and floral parts of herbs and trees. The adults may be found from September until the first frosts in November or December. Young prereproductive adults can best be observed from September through October.

Observations were initiated in October 1989 in mesquite grasslands north of Madera Canyon and at the Buenos Aries nature reserve in south Arizona. The study was continued in October 1990 in the *Acacia constricta* grasslands near Portal in S.E. Arizona. There was no rain or cloud cover on 5 of the observation days at the Portal site. The remaining 3 had cloud cover and sometimes rain for at least half of the observation period, in each case including the midday roosting period, and these are characterised as "overcast days" (Table 1).

A total of 205 h of observations provided the data for analysis. This included full day observations of 13 different insects (Table 1), and shorter observations ranging from 20 to 404 minutes on an additional 29. The comments matching plant identity with feeding events were lost for two full-day insects (insect 11 and 12), and these were therefore excluded from certain analyses (e.g., Tables

2, 6, 7 and 8) but could be included in others (e.g., overall activity budgets and activity patterns).

Data recording

Shortly after sunset of the day before observations, roosting insects were marked on the thorax with a non-toxic Speedball^{R} paint marker pen. This allowed approximately 12 h for recovery from disturbance before observations. Where possible, five insects on a single tree were marked with different colours. The first of these to leave the roost and begin foraging was selected and observed throughout the day until the initiation of its overnight roost. The reason for marking several insects was, firstly, to ensure that at least one could be located the following morning; in reality, the insects moved little during the night and could all be located the following morning in the same roost, often in the same position they had been left the previous evening. Secondly, marking several insects avoided the potential situation in which an only insect marked would not leave the roost to feed due to, for example, an initial disturbance by the observer, illness, phase of the lifecycle (e.g., preoviposition insects) etc. On one occasion, the observer lost sight of the observation insect after the remaining marked insects had left the roost. An unmarked insect (number 13) was therefore observed for the morning foraging period, after which an attempt was made to mark the

insect in the hope that the disturbance would subside during the midday roosting period. The insect locomoted little and did not feed in the afternoon, and it is suspected this may be the result of being handled.

Insects were observed using binoculars where necessary, and behaviour was recorded on a hand-held Hewlett Packard HP71B electronic event recorder. The following events were recorded:

1) The durations of periods of locomotion and quiescence and whether the insects were on the ground or in trees or shrubs.

2) The durations of feeding bouts and the nature of the food item. Where plants were the food items, it was noted whether the leaves, stem or reproductive parts were being eaten. Plants were number-coded and marked with plastic marker flags for later collection and identification. The nomenclature of Leher (1978) was followed.

Feeding was recognised by mandibular movement. However, in some instances it was clear that this behaviour was not accompanied by ingesting. For example, insect no. 8 spent 4.2 minutes chewing on a nylon camera bag and in the same "meal", 7.5 mins chewing on the surface of a dead, hardened seedpod (see Fig. 5). Subsequent inspection of these items showed no sign of material having been removed, and these were therefore not included as feeding events in analyses.

3) Rejections of potential food items and whether or not

they occurred following palpation or biting. The data were later edited to remove redundancies in the hierarchical sequence of behaviours associated with feeding. Thus, where an encounter led to feeding, the preceding palpations and bites were removed from the data set; likewise, where a food item was rejected after biting, the preceding palpations were edited out. Where rejections were associated with more than one bite or palpation, only one was retained to represent the outcome of an encounter. In this way each encounter with a potential food item was represented in the data set as a single behaviour, which was the highest level in the hierarchy of feeding behaviours to which the encounter led. This enabled the use of the number of palpations and bites as a quantitative measure of rejection rates (e.g., Table 5).

Definition of foraging periods

Insects observed on sunny days fed during two distinct periods, one in the morning and one in the afternoon, separated by an extended period of roosting (Fig. 4 and 6). These periods in which feeding occurred are henceforth referred to as foraging periods. Foraging periods were defined, for the purposes of data analysis (e.g., Table 2), to begin with the bout of locomotion immediately preceding the insect's first bite or feed on a potential food item after leaving the roost. Foraging periods ended after the

final feed or rejection prior to ascending the roost at midday or in the evening.

Bout criteria

Log-survivor curves (Simpson 1982) were used to investigate whether behaviours separated into distinct populations with regard to their duration. It is preferable, where possible, to derive separate bout criteria for individual insects. This is because, when pooled data are used, a point of inflection (representing a bout criterion) could distinguish sub-populations of insects rather than categories of behaviour within insects. Also, variability among insects may obscure points of inflection, hiding bout criteria which are apparent in plots for individuals. However, low numbers of data points for individual insects meant that it was often not possible to determine with confidence the location of points of inflection. Therefore, pooled data were used, but only after it had been established from the data for individuals that the insects did not separate out into sub-populations with respect to the occurrence and location of bout criteria. In practice, pooled data for interfeed periods (Fig. 1) and feeding periods (Fig. 2) showed clearly delineated points of inflection which were also apparent in approximate location in individual insects. For periods of quiescence, there were distinct breaks at 0.01, 0.02 and 43 minutes (Fig 3). There were also points of inflection at

approximately 1.4 and 3 minutes, but these were less distinct in the plot of pooled data due to greater variability among insects.

A second use of log-survivor curves was to recognise recording artifacts due to the upper limit in resolution possible under the circumstances of the present study. The data were processed on an electronic spreadsheet, and the algorithm used calculated periods of quiescence by subtracting the time at the end of a behaviour from that at the start of the following behaviour. Whereas the transition between behaviours is often continuous (e.g., an insect may stop walking and start feeding simultaneously), it cannot be recorded as such and there are therefore short pauses in the data between the termination of one behaviour and the start of another. Such pauses may be expected to fall into two categories: very brief ones, as the observer's finger was moved from the current position to the appropriate key on the data recorder, and slightly longer ones in which it was not immediately apparent which behaviour to record. The inset to Figure 3 shows that there were sharp points of inflection at 0.01 and 0.02 minutes, and these are considered to represent the two categories of artefact. Evidence that these breaks represent recording artifacts is provided by their distinctness in plots of pooled data, as compared with the less distinct breaks representing insect behaviour which is subject to individual variation. Periods

of quiescence of 0.02 minutes or shorter were therefore not included in further analyses. This is particularly important in detailed analysis of activity budgets (e.g., Table 2), especially for calculations involving the number of quiescent periods.

RESULTS

Time budgets

Table 1 shows the time budgets for the 13 insect that were observed continuously throughout the day, together with the description of each insect (population, and whether they were observed on sunny or overcast days). Insect number 13 was considered abnormal on account of possible disturbance (see Methods), and was therefore excluded from the calculation of statistics and tests which follow. It can be seen that the major portion of the day was spent in quiescence (mean$\pm$SE% = 75.3$\pm$2.8), followed by locomotion (17.7$\pm$2.3) and feeding (6.9$\pm$1.1). The percentage of the day spent feeding was similar in insects observed on sunny (7.0$\pm$1.1) and overcast (6.6$\pm$1.3) days ($t_{(df 10)}$=0.05, p=0.96; this and the two tests which follow were performed on arcsine-square root transformed proportions). However, the insects observed on overcast days spent a significantly larger proportion of the day locomoting than those on sunny days (27.0$\pm$1.1 and 14.6$\pm$2.2 for overcast and sunny days

Table 1. Time budgets of 13 adult female *T. eques* observed continuously throughout the day

Insect no.	Description	% feed	% loc.	% quies.	Observed (mins)
1	Portal, rain	6.8	26.9	66.3	627
2	Portal, rain	4.2	28.9	67.0	604
3	Portal, rain	8.8	25.1	66.1	657
4	Portal, sun	3.2	21.1	75.7	671
5	Portal, sun	0.7	7.7	91.6	689
6	Portal, sun	12.5	15.5	72.0	657
7	Portal, sun	12.7	28.8	58.5	627
8	Portal, sun	6.6	14.0	77.6	656
9	Madera, sun	6.9	11.2	81.9	630
10	Madera, sun	4.5	12.5	83.1	665
11	Buenos aries, sun1	11.2	11.3	77.5	553
12	Buenos aries, sun1,2	4.6	9.1	86.3	607
13	Madera, sun3	1.7	10.9	87.4	537

1. recorded comments matching plants to feeds lost
2. only fed in the afternoon foraging period
3. only fed in the morning foraging period

respectively; $t_{(df10)}=3.03$, $p=0.013$). Conversely, the sunny-day insects spent a larger proportion of the day quiescent (78.2±3.2) than those observed on overcast days (66.5±0.27; $t_{(df8.1)}=3.66$, $p=0.006$ - owing to inequality of variances, separate variance estimates were used).

Insects observed on sunny days had two foraging periods (see Methods for definition) separated by an extended interfeed over the midday period (see Figs 4 and 6). Table 2 presents a comparison of time budgets in the morning and afternoon foraging periods. Insects fed and locomoted for significantly longer during the morning foraging periods, with both these increased times being accounted for by a greater number rather than an increased duration of individual feeding or locomoting events. There was, additionally, a greater number of quiescent periods in the mornings, but due to high variances the total time spent quiescent was not significantly greater in the morning than the afternoon. However, the proportion of time spent feeding, locomoting and quiescent was similar in the two foraging periods (see percentage scores in Table 2), with the differences being accounted for by a significantly longer period spent foraging in the mornings.

Patterning of activities

a. *Meals, feeds and interfeeds*

To investigate whether feeding events occurred randomly

Table 2. Comparison of feeding, locomotion and quiescence in the morning and afternoon foraging periods of *T. eques* in the field. N = 7: only insects with distinct morning and afternoon foraging periods (i.e., those observed on sunny days) are considered; two insects for which comments were lost, and one which did not feed in the afternoon are ommitted (see Table 1). The data represent means ± SE (all, except for counts, in minutes). Significance levels refer to a two-tailed t-test for matched pairs.

(a.)	Morning	Afternoon	Sign.
Duration of foraging period	166.6±21.9	99.4±18.3	0.016*
Feed			
No.	37.0±8.5	19.9±5.7	0.029*
Avg. duration	0.82±0.19	0.74±0.16	0.420
Total time	31.3±7.8	13.8±3.8	0.019*
% time	17.6±3.8	14.8±4.6	0.540
Locomote			
No.	88.4±18.1	46.1±13.5	0.011*
Avg. duration	0.76±0.11	1.3±0.51	0.335
Total time	57.9±8.4	36.1±8.9	0.001**
% time	36.2±3.7	38.6±6.9	0.800
Quiescence			
No.	135.0±32.5	61.3±18.4	0.021*
Avg. duration	0.77±0.21	2.1±1.2	0.340
Total time	77.5±11.6	49.5±12.6	0.191
% time	46.1±3.3	46.5±8.4	0.970

within foraging periods or were clustered into bouts, a log-survivor curve (Simpson 1982) for the duration of interfeed intervals was plotted (Fig. 1). From the figure it can be seen that there were two distinct points of inflection, representing bout criteria at 8 and at 49 minutes. The smaller of these indicates that feeding was clustered into bouts in which individual feeds were separated by brief periods of locomotion and quiescence of less than 8 minutes in total. These bouts, henceforth referred to as meals, were separated by longer periods of 8 minutes or more in which the insects did not feed. The patterning of meals over the day for the full-day insects 1 to 12 can be seen in Fig. 4, while Fig. 5 shows selected examples of the distribution of feeds and rejections within meals.

The 49 minute bout criterion suggests that meals were themselves clustered into bouts, which were separated by non-feeding periods of more than 49 minutes. In insects observed on sunny days, these longer periods without feeding ranged from 76 to 550 minutes, and invariably coincided with the extended midday roosts (Figs 4 and 6). They were, however, not considered a suitable criterion in defining foraging periods (see Methods), since insects often continued to sample and reject plants after the last feed in the morning period, and started sampling before the first feed following the midday roost. While not actually feeding, such sampling is considered to be foraging behaviour.

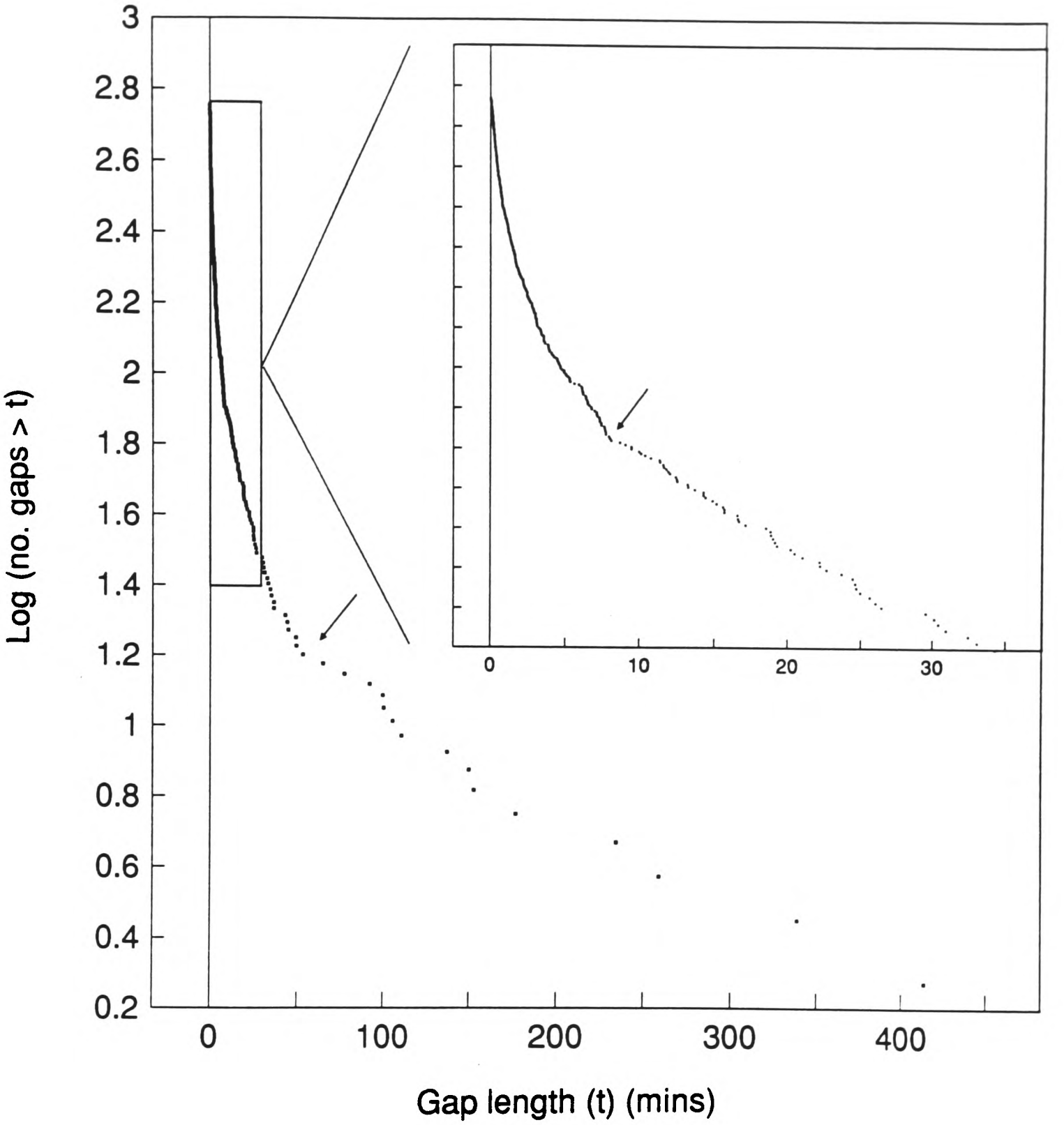


Figure 1. Log-survivorship plot for interfeed intervals of 13 *T. eques* observed continuously throughout the day.

By contrast, on overcast days the interfeed intervals longer than 49 minutes tended to be shorter (ranging from 64 to 180 mins) and occurred more frequently (Fig. 6). In addition, comparison of the plots for sunny and overcast day insects in Fig. 6 suggests that a larger proportion of the intermeal intervals was spent locomoting on overcast than sunny days. This was confirmed by a t-test in which the proportion of time spent locomoting during intermeal intervals by insects 1, 2 and 3 ($X \pm SE\% = 27.0 \pm 0.6$) was tested against that for sunny day insects (13.5 ± 2.2; $t_{(5.32df)}=5.32$, $p<0.001$ using separate variance estimates on arcsine-square root transformed proportions).

A log-survivor plot of duration of individual feeding events showed a point of inflection at 0.15 minutes, and another at 0.29 minutes (Fig. 2). This suggests that there were two classes of feed which differed from the more sustained feeding events of longer duration (>0.29 mins). These brief feeding events, henceforth referred to as "nibbles" (<0.15 mins) and "snacks" (between 0.15 and 0.29 mins), warrant special attention in the determination of feeding patterns. Both nibbles and snacks were frequently embedded within meals (e.g., Fig. 5) and were therefore considered a part of meal taking. However, a nibble or snack on its own (i.e., separated from other feeds by 8 mins or more) cannot reasonably be considered equivalent to a meal. Such events were therefore taken to be forms of rejection

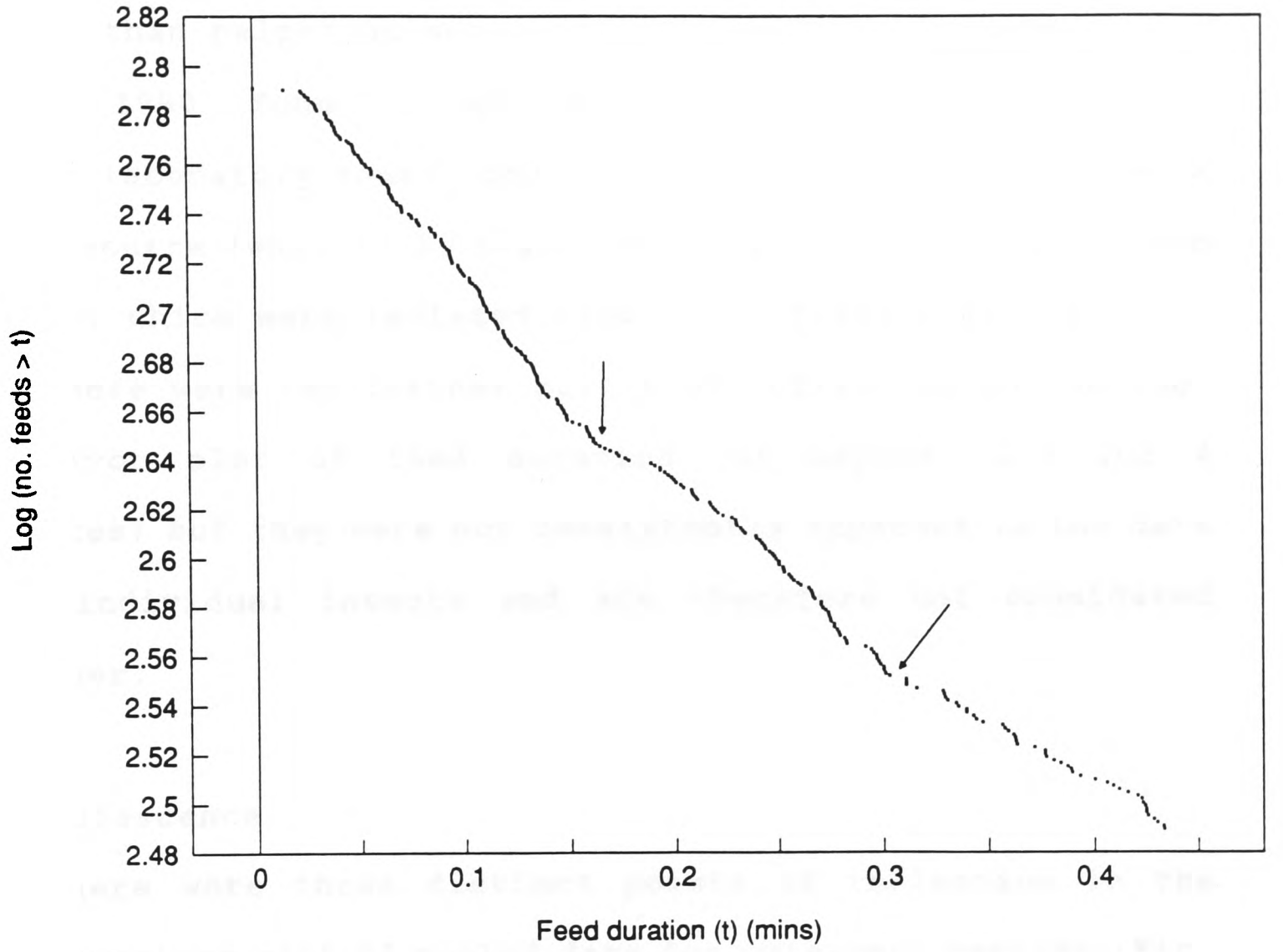


Figure 2. Log-survivorship plot for feed durations of 13 *T. eques* observed continuously throughout the day.

behaviour and not included in the determination of intermeal intervals (Fig. 6; Table 4). In a laboratory study of *Locusta migratoria* and *Schistocerca gregaria*, Blaney et al. (1985) distinguished 3 categories of rejection behaviour other than palpating and biting. Similarly, Simpson (1981, 1982, 1990) found using log-survivor analysis that even under laboratory conditions with a single, highly palatable food source (wheat), *L. migratoria* took nibbles of less than 1 min. which were isolated from other feeding events.

There were two further points of inflection in the log-survivor plot of feed duration (at approx. 1.9 and 4 minutes) but they were not consistently apparent in the data for individual insects and are therefore not considered further.

b. *Quiescence*

There were three distinct points of inflection in the log-survivor plot of pooled data for quiescent periods (Fig. 3). The first and second bout criteria occurred at 0.01 and 0.02 minutes. These, as discussed above, represent artifacts due to the maximum resolution of the data recording technique. The third bout criterion occurred at 43 minutes. As discussed below, this primarily distinguished the insects observed on sunny days from those observed on overcast days.

In addition to these bout criteria, data for individual

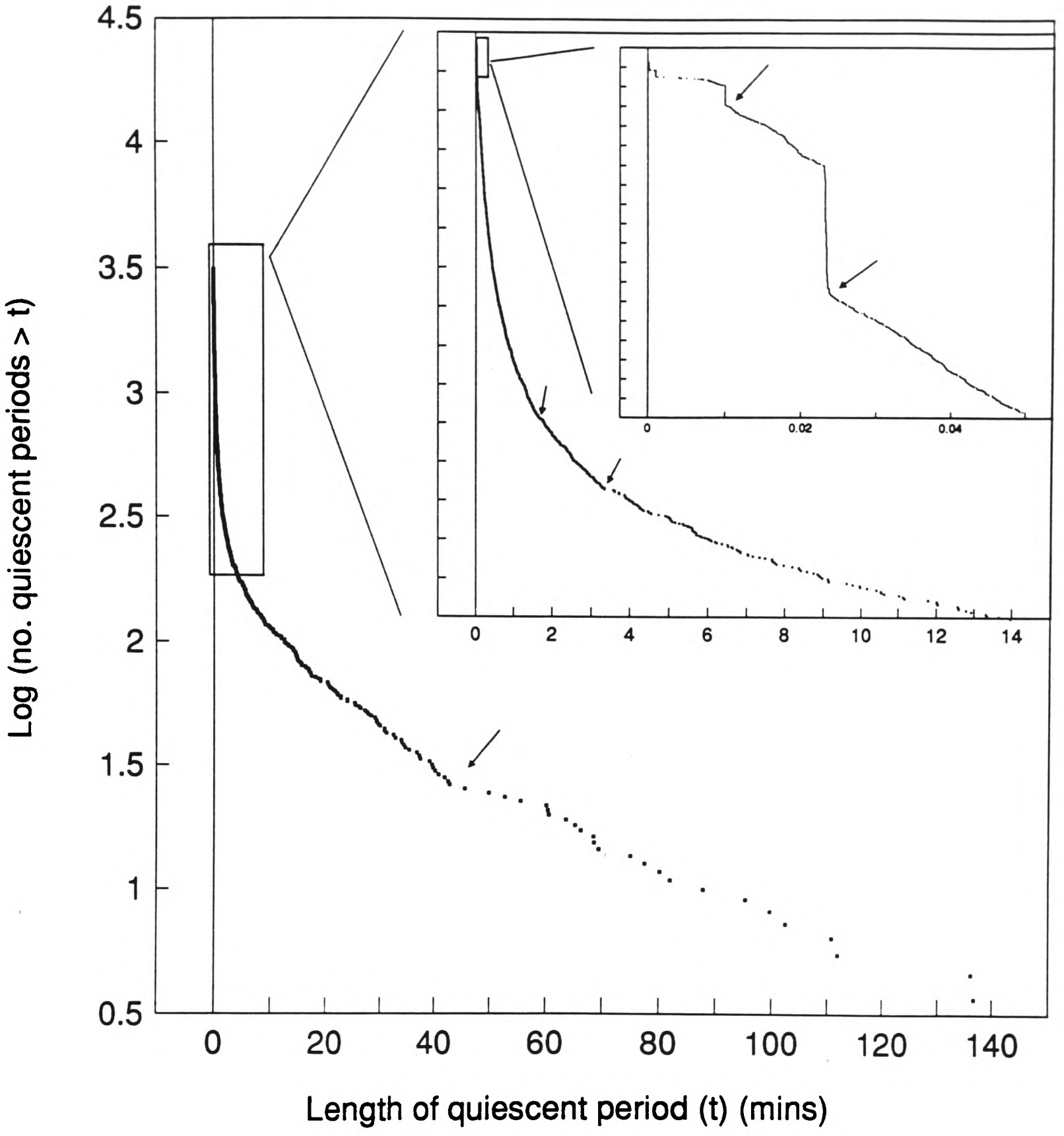


Figure 3. Log-survivorship plot for quiescent periods of 13 *T. eques* observed continuously throughout the day.

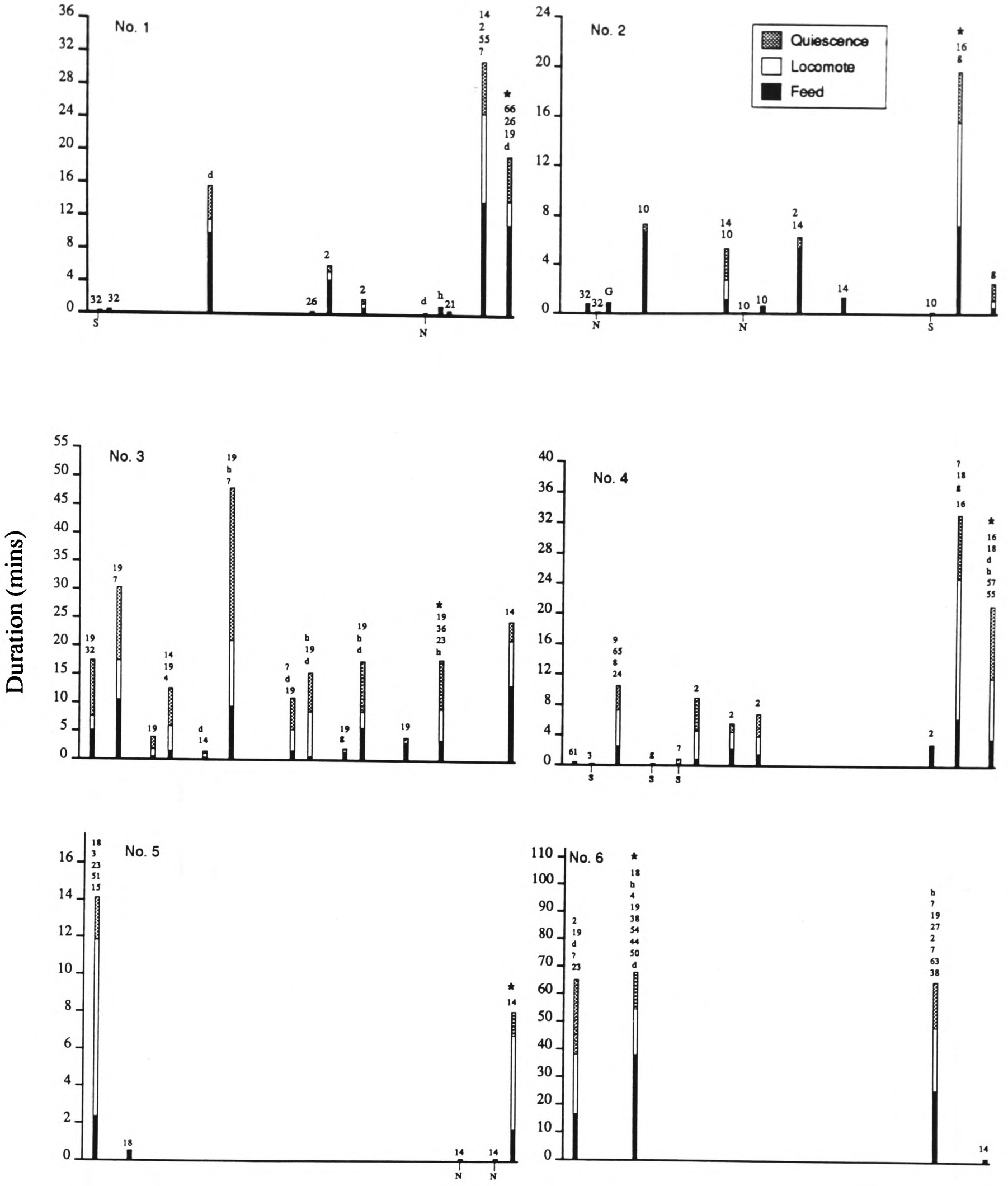
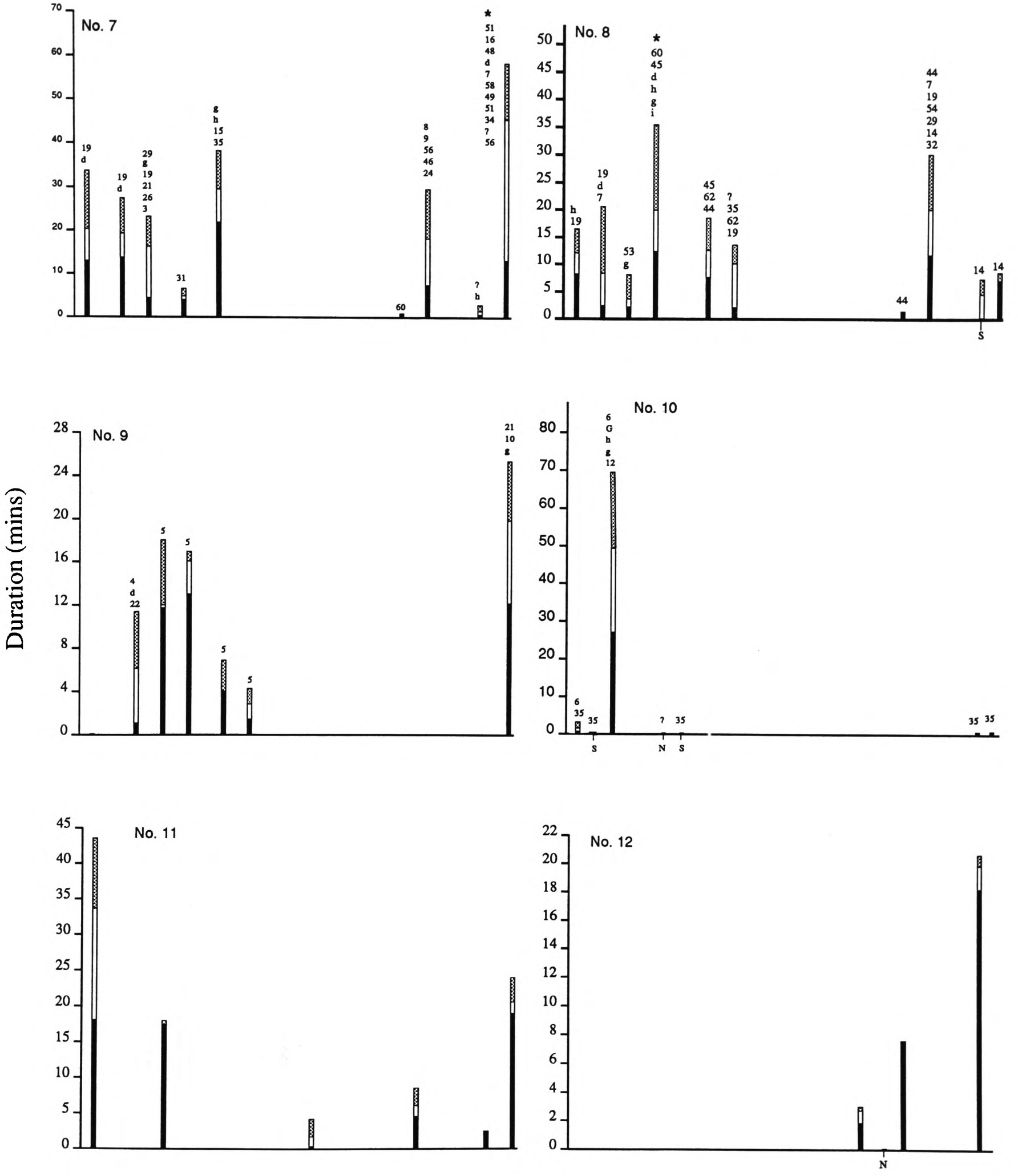


Figure 4. Pattern of meals of 12 *T. eques* observed continuously throughout the day (see overleaf for insects 7-12). Insects 1 to 3 were observed on overcast days and the rest on sunny days. The height of bars indicates meal duration, and the stacks within bars time spent feeding, locomoting and quiescent within meals. An asterisk above the vertical bars identifies individual meals which are detailed in Figure 5. Other symbols above vertical bars refer to the items eaten in each meal, and correspond with the codes in Table 5. Records matching foodplants with feeding events were lost for insects 11 and 12. The symbols S and N along the horizontal axis signify brief periods of feeding termed "snacks" (between 0.15 and 0.29 min.) and "nibbles" (<0.15 min. - see text for further explanation). In each case, the horizontal axis represents approximately 600 minutes.


Duration (mins)
No. 7
19 d
19 d
29 g 19 21 26 3
31
g h 15 35
60
8 9 56 46 24
? h
* 51 16 48 d 7 58 49 51 34 ? 56
No. 8
h 19
19 d 7
53 g
* 60 45 d h g i
45 62 44
? 35 62 19
44
44 7 19 54 29 14 32
14
S
14
No. 9
4 d 22
5
5
5
5
21 10 g
No. 10
6 35
35
6 G h g 12
? 35
S N S
35 35
No. 11
No. 12
N

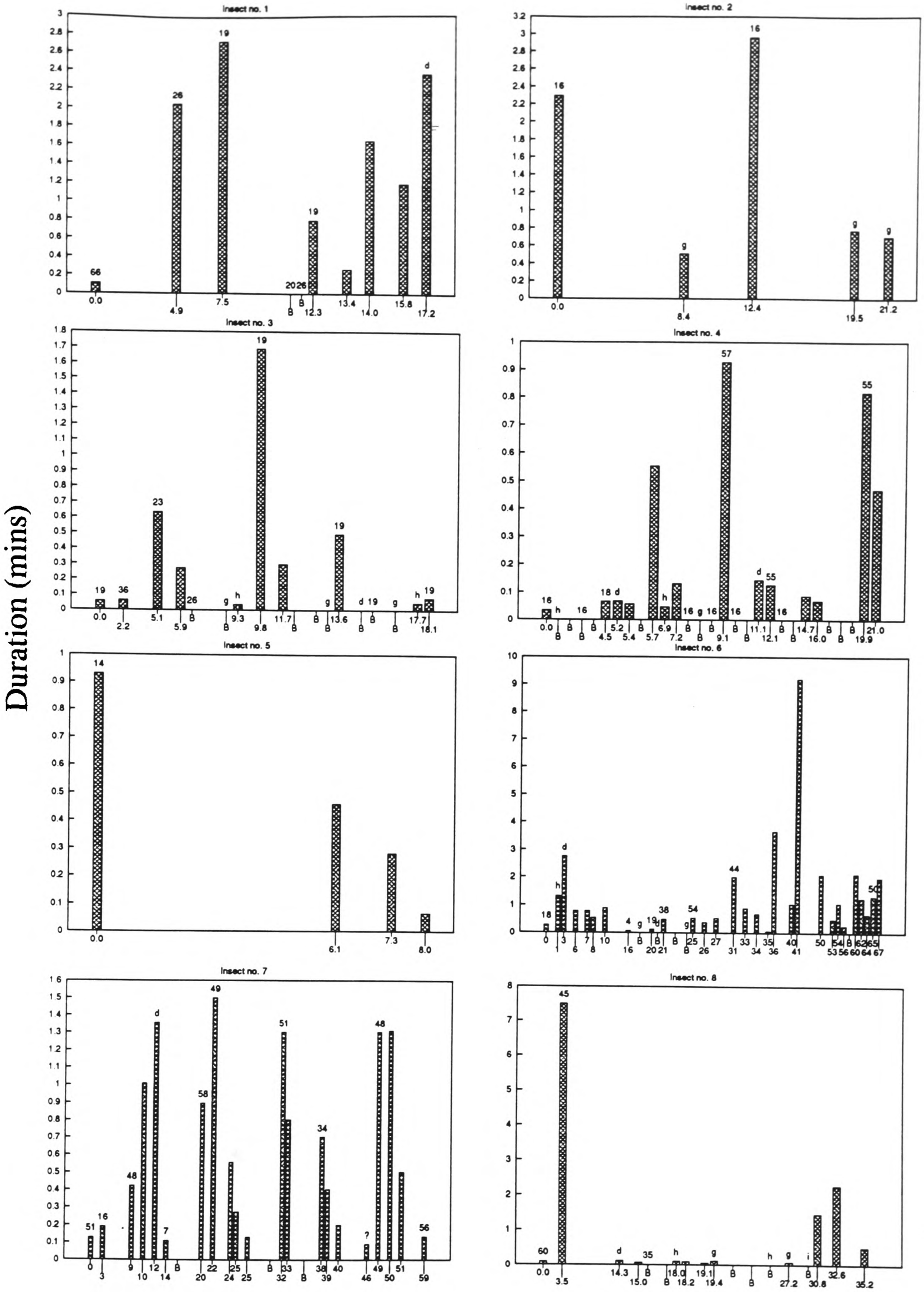


Figure 5. Examples of patterns of feeds within meals. "B" on the horizontal axis signifies rejection at the level of biting. The letters above bars identify the food items and correspond with the codes in Table 5. When a continuous sequence of feeds and/or bites is on the same food item, only the first of these is coded.

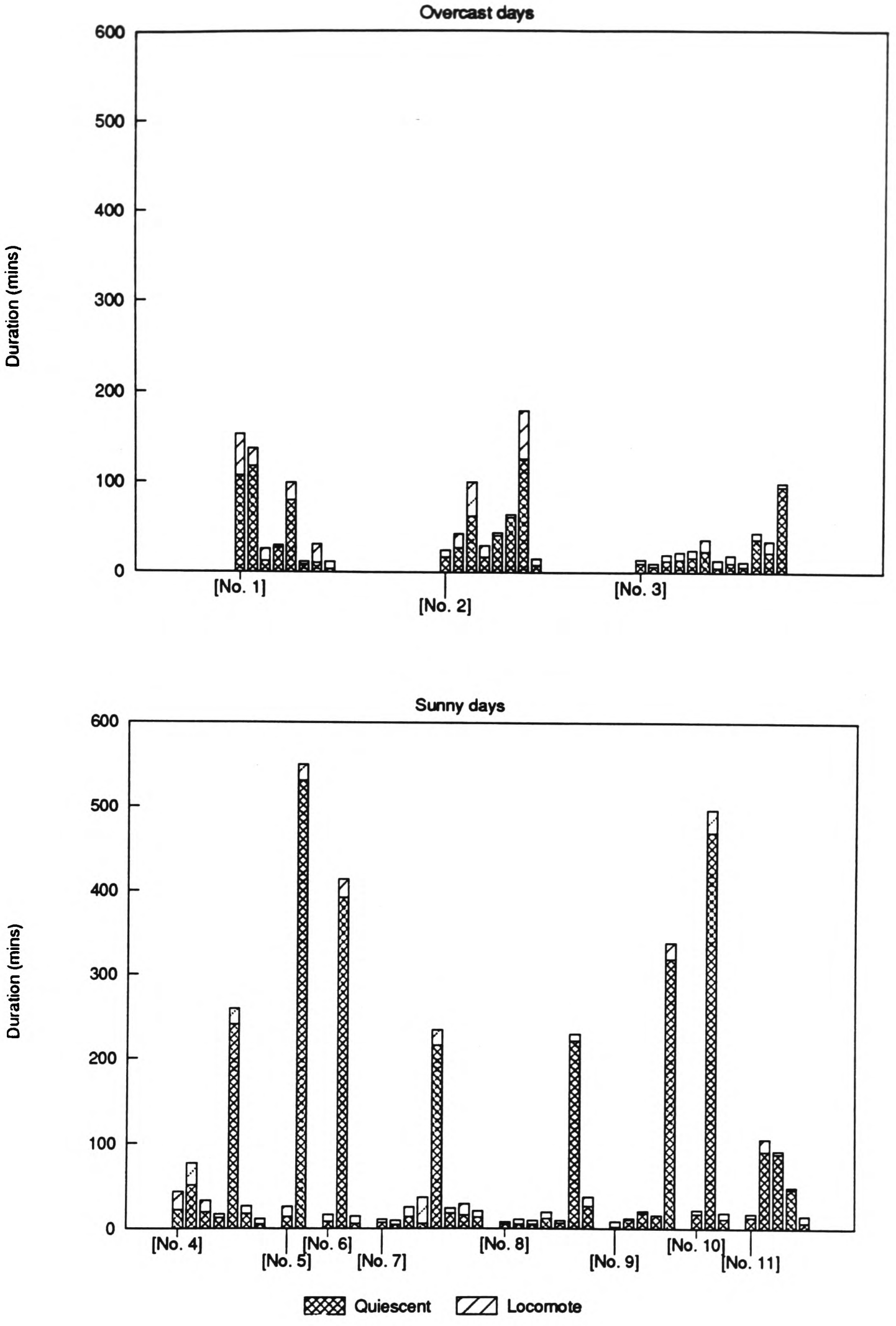


Figure 6. Duration of intermeal intervals of insects observed on overcast and sunny days. Bar height signifies total duration, and the stacks represent time quiescent and locomoting within the intermeal intervals. Bars are placed in order of occurrence.

insects suggested that there were further points of inflection round 1.4 and 3 minutes. Owing to greater variability among insects these were, however, less distinct in the plot of pooled data. Inspection of the data suggested that quiescent periods of intermediate duration (between 1.4 and 3 mins) were particularly common within meals and those of longer duration occurred almost exclusively between meals. A test of association between these categories of quiescent period and whether they occurred within or between meals should, however, exclude all periods of quiescence with duration 8 minutes or longer. This is because quiescent periods within a meal cannot exceed 8 minutes (8 minutes of not feeding terminates a meal), while quiescent periods between meals may vary freely. Table 3a shows such a test, which demonstrates a highly significant association between short quiescent periods and meals, and longer quiescent periods and intermeal intervals.

Simpson (1990) has stressed the advantages, when using log-survivor analyses to distinguish intra- from intermeal gaps, of identifying behavioural correlates for any bout criterion. In *Locusta migratoria* under laboratory conditions, gaps exceeding the bout criterion were accompanied, in more than 90% of cases, by the insects leaving the food after feeding and entering a characteristic resting position on a perch (Simpson 1990). It was therefore of interest to determine in the present study the proportion

Table 3. Table of association between the duration of quiescent periods (t) and whether they occur (a.) within or between meals and (b.) on sunny or overcast days. In (a.), two categories of quiescent period are recognised according to the bout criteria established using log-survivor curves (see Fig 3): those between 1.4 and 3.0 mins, and those greater than or equal to 3 mins. Only periods less than 8 mins are considered because, by the definition of intermeal intervals, quiescent periods within meals cannot exceed 8 mins (see text for further explanation). In (b.), two categories are tested: those greater than and those less than or equal to 43 mins. Cell contents are as in the top left hand corner of the table.

(a.)

Count Exp Val Residual Std Res	Between meals	Within meals	Row Total
1.4<t<3.0	104 116.7 -12.7 -1.2	35 22.3 12.7 2.7	139 58.6%
3.0<t<8.0	95 82.3 12.7 1.4	3 15.7 -12.7 -3.2	98 41.4%
Column Total	199 84.0%	38 16.0%	237 100.0%

Chi Square	D.F.	Sign.
19.28	1	p< 0.0001

(b.)

Count Exp Val Residual Std Res	Rain	Sun	Row Total
t>43	1 9.0 -8.0 -2.7	27 19.0 8.0 1.8	28 6.0%
t<43	149 141.0 8.0 0.7	290 298.0 -8.0 -0.5	439 94.0%
Column Total	150 32.1%	317 67.9%	467 100.0%

Chi Square	D.F.	Sign.
11.13	1	p< 0.0008

of intermeal gaps in which the insects took at least one unbroken period of quiescence exceeding the 3 minute criterion discussed above (Fig. 3; in what follows, these periods are referred to as "postprandial rests"). Of 70 intermeal gaps (i.e., gaps exceeding the 8 min. bout criterion), 79% (55) contained at least one postprandial rest ($p=0.003$ using Wilcoxon's signed ranks test in which the number of gaps containing was paired for each insect with the number lacking postprandial rests). Of the 55 postprandial rests scored, all but two took place in a characteristic vertical perching position on the aboveground parts of bushes or trees.

The data for individual insects suggested that quiescent periods of duration exceeding the 43 minute bout criterion (Fig. 3) occurred mostly in insects observed on sunny days. Table 3b shows that there was a highly significant association between long periods of quiescence and sunny days. From the table, it can be seen that the greatest contribution to the Chi Square value was due to the lower than expected number of long periods of quiescence on overcast days (7.3 out of a Chi Square of 11.13; determined by squaring the standardized residual for this cell). The second greatest contribution (3.2) was due to the greater than expected number of long periods on sunny days, while the quiescent periods of shorter duration were distributed across overcast and sunny days approximately as expected

(together they contribute only 0.74 to the total Chi Square value). Therefore, the weather conditions appear to have affected predominantly the quiescent periods of longer duration. This suggests that the greater proportion of quiescence in the time budgets of insects observed on sunny days (see above) can be accounted for by a tendency to take longer periods of unbroken quiescence than those observed on overcast days.

Pre and postprandial correlations

It has been observed in laboratory studies of insects (Simpson 1982; Simpson et al. 1989; Reynolds et al. 1986; Bowdan 1988; Chapman & Beerling 1990) that relationships may exist between meal size and various components (locomotion, quiescence, total duration) of the intermeal interval preceding (preprandial correlation) or following (postprandial correlation) the meal. To investigate this for the current data, the product-moment correlation coefficient was computed for individual insects for the relationship between intermeal intervals preceding and following meals, and the duration and estimated size (time spent feeding within a meal) of the meals (see Table 4). For each correlation, the mean of all insects was computed and tested against zero using a one-sample t-test; a significant positive value would suggest a tendency for the correlation variables to be positively related, and vice-versa.

Table 4 shows that the time spent locomoting in intermeal

Table 4. Relationship between (a.) meal size (approximated by time spent feeding within a meal) and (b.) meal duration and time spent locomoting, quiescent, and total duration of the intermeal interval preceding (preprandial) and following (postprandial) the meal. Correlation coefficients were obtained individually for 8 insects, and transformed to approximate a normal distribution using Fisher's transformation for correlation coefficients (Rohlf and Sokal 1981). The means of the transformed data were tested in a 2-tailed one sample t-test against 0.

	Preprandial			Postprandial		
	Locomote	Quies.	Total	Locomote	Quies.	Total
a. Feed duration						
mean	0.423	-0.118	0.054	-0.274	-0.198	-0.257
S.E.	0.158	0.244	0.246	0.073	0.199	0.192
t	2.47	-0.68	0.34	-3.45	-1.13	-1.42
Prob. of t	0.043*	0.518	0.743	0.010**	0.297	0.198
b. Meal duration						
mean	0.245	-0.280	-0.171	-0.134	-0.237	-0.273
S.E.	0.162	0.185	0.200	0.087	0.153	0.152
t	1.61	-1.40	-0.98	-1.56	-1.58	-1.88
Prob. of t	0.151	0.204	0.358	0.162	0.158	0.102

intervals was positively related to the time spent feeding in subsequent meals. By contrast, time spent locomoting in intermeals was negatively related to the time spent feeding in the preceding meal. There were no significant correlations involving meal duration, time spent quiescent in intermeals or total duration of intermeal intervals.

Polyphagy

a. *Host range*

Insects were observed to feed on 61 different items, and contacted but rejected a further 14; these are almost certainly underestimates since they do not include a number of unidentified plants and other items. Potential food items on which palpations, bites or feeds were recorded are listed in Table 5, together with total times spent feeding on each, average feed durations, number of feeds, number of contacts, number of insects that contacted each and an index of acceptability (number of feeds/number of contacts). The number of contacts can be taken as a measure of confidence in the validity of the preference and acceptability indices, since the outcomes of several contacts provide a more reliable estimate than from a single contact. For example, a single contact resulting in a feed would yield the highest possible acceptability index (1), yet we would have to be more confident in the acceptability of a plant which was fed on 9 times out of 10 contacts, even though the acceptability

Table 5. Acceptability ranking of food items eaten by *T. eques* in the field. Data for food items which were contacted by 2 or fewer insects (b.) are considered less reliable than those contacted by more (a.) and are therefore presented separately. Entries without details of plant part refer to leaves. "Code" pertains to Figures 4 and 5. Food items which were not identified plants are indented. For (a.), data are ranked in descending order of the acceptability index (NF/NC).

a.) Contacts by more than 2 insects

Code	Plant species/ food item	Time feeding (TF) (min.)	No. feeds (NF)	No. contacts (NC)	NF/ NC	TF/ NF	No. insects
1	Euphorbia hyssopifolia	42.2	27	31	0.92	1.60	11
2	Acacia constricta flowers	30.0	40	46	0.91	1.07	4
3	Allionia incarnata	1.8	9	12	0.77	0.19	5
4	Eragrostis cilianensis seeds	0.4	3	4	0.75	0.09	4
5	Isocoma tenuisectus flowers	82.9	51	62	0.75	1.40	9
6	Eragrostis lehmannii seeds	26.4	21	26	0.74	1.42	6
7	Talinum aurantiacum	3.1	5	9	0.73	0.56	4
8	Dead insects	1.6	2	3	0.67	0.52	3
9	Sida abutifolia	0.5	2	3	0.67	0.17	3
10	Molluga verticillata	21.3	20	25	0.66	0.71	7
h	Unidentified herbs	19.6	32	51	0.65	0.56	11
12	Euphorbia florida	37.7	30	39	0.62	0.80	7
13	Salsola kali	14.3	11	17	0.58	0.92	3
14	Acacia constricta	37.9	60	82	0.55	0.61	10
15	Gutierrezia sorothrae	9.7	6	12	0.52	0.82	4
16	Acourtia nana	10.5	12	29	0.51	1.13	3
17	Ambrosia sp.	1.4	10	15	0.50	0.10	4
18	Bouteloua barbata seeds	2.2	12	21	0.50	0.16	7
19	Portulaca oleraceae	112.3	168	352	0.50	0.64	6
G	Unidentified grass seeds	1.7	3	6	0.50	0.33	5
21	Solanum eleaginifolium	3.4	4	9	0.48	0.81	4
22	Chamaecrista leptidenia	4.6	8	16	0.42	0.39	5
23	Acacia constricta seedling	1.4	5	19	0.41	0.16	6
24	Hilaria mutica	0.7	3	8	0.39	0.25	3
?	Unidentified items	37.5	42	111	0.35	0.41	15
26	Trianthema portulacastrum	2.4	3	14	0.33	0.31	4
27	Cow dung	0.1	1	3	0.33	0.04	3
g	Unidentified grasses	25.3	52	154	0.33	0.31	23
29	Gutierrezia sp.	2.0	1	4	0.33	0.68	3
30	Bouteloua gracilis	0.9	1	3	0.33	0.31	3
31	Isocoma tenuisecta	2.3	6	16	0.27	0.10	8
32	Acacia constricta bark	2.0	7	27	0.21	0.16	7
d	Debris	23.9	43	237	0.20	0.29	17
34	Prosopis juliflora bark	1.3	3	9	0.19	0.11	4
35	Prosopis juliflora	19.0	15	42	0.19	0.23	13
36	Guilleminea densa	0.1	1	8	0.11	0.02	3
i	Inorganic items	4.2	3	11	0.08	0.23	6
38	Chloris virgata	0.5	2	13	0.07	0.09	3
39	Bouteloua barbata	0.0	0	27	0.00	0.00	7
40	Tidestromia lanuginosa	0.0	0	10	0.00	0.00	4
41	Eragrostis lehmannii	0.0	0	37	0.00	0.00	9
42	Bouteloua aristoides	0.0	0	17	0.00	0.00	3
43	Ambrosia confertiflora	0.0	0	7	0.00	0.00	5

Table 5. contd.

b.) Contacts by 2 or fewer insects

Code	Plant species/ food item	Time feeding (TF) (min.)	No. feeds (NF)	No. contacts (NC)	NF/ NC	TF/ NF	No. insects
44	Ephedra trifuria	33.5	21	28	0.83	1.49	2
45	Hardened dead seed pod	8.7	3	3	1.00	2.91	1
46	Muhlenbergia porteri	6.3	6	8	0.75	1.04	1
47	Diodea teres	6.1	3	3	1.00	1.88	2
48	Croton corymbulosus	4.5	5	6	0.83	0.91	1
49	Erioganum wrightii flowers	3.4	4	4	1.00	0.84	1
50	Leptochloa dubia	3.3	2	3	0.67	1.65	1
51	Euphorbia serpyllifolia	2.8	5	6	0.88	0.50	2
52	Eragrostis intermedia	2.6	4	5	0.83	0.64	2
53	Opuntia sp.	2.1	4	4	1.00	0.53	1
54	Boerhaavia sp.	2.1	5	5	1.00	0.40	2
55	Boerhaavia intermedia	1.8	6	9	0.75	0.30	2
56	Euphorbia albomarginata	1.0	2	2	0.49	0.49	1
57	Rhyncosida physocalyx	0.9	1	2	0.50	0.47	2
58	Erioganum wrightii	0.9	1	2	0.50	0.89	1
59	Sida spinosa	0.7	2	2	1.00	0.36	1
60	Prosopis seedling	0.5	3	3	1.00	0.16	2
61	Sphaeralcea sp.	0.4	1	4	0.17	0.22	2
62	Argythamnia neomexicana	0.3	2	2	1.00	0.15	1
63	Malvella lepidota	0.2	2	13	0.14	0.05	2
64	Boerhaavia spicata	0.2	1	4	0.17	0.10	2
65	Parthenium incanum	0.2	1	5	0.17	0.09	2
66	Portulaca parvula/mundula	0.1	1	3	0.50	0.06	2
67	Erioneuron pulchellum	0.0	0	4	0.00	0.00	1
68	Amaranthus palmeri	0.0	0	2	0.00	0.00	2
69	Brachiaria arizonica	0.0	0	2	0.00	0.00	1
70	Cenchrus incertus	0.0	0	1	0.00	0.00	1
71	Bouteloua curtipendula	0.0	0	22	0.00	0.00	1
72	Panicum hirticaule	0.0	0	1	0.00	0.00	1
73	Eragrostis cilianensis	0.0	0	1	0.00	0.00	1
74	Aristida adscensionis	0.0	0	1	0.00	0.00	1
75	Zinnia grandiflora	0.0	0	1	0.00	0.00	1

index would have a lower value (0.9). Potential foodplants (i.e., those which were contacted) included 53 species from 42 genera, spanning a range of 16 plant families (Table 6a). Of these, 43 species in 34 genera were eaten to some degree. However, feeding time was not distributed evenly, with five plant families being dominant: Asteraceae (16.3%), Euphorbiaceae (12.9%), Fabaceae (15.8%), Poaceae (10.5%) and Portulacaceae (17.2%).

Table 6b shows the proportions of total feeding time on the major food categories. The leaves of herbaceous plants were the major foods, constituting 43.1% of total feeding time and 44.9% of the total number of feeds. Following this were the flowers of woody plants, on which 18.2% of feeding time was spent, constituting 11.6% of the total number of meals. That the percentage of time feeding on flowers of woody plants is a third greater than the percentage number of feeds, indicates that on average there were larger meals than on the leaves of herbs where the percentage ratio was almost unity. The leaves and reproductive parts of grasses were represented equally in the percentage time feeding, but the higher number of feeds on grass leaves suggests that feeds on these were on average shorter than on reproductive parts. Detailed vegetation analyses were not carried out because individual insects encountered plants at a scale considered more fine-grained than could justifiably be represented by general vegetation surveys. However, it is

Table 6. The representation of (a.) plant families and (b.) major food categories in the diets of adult female *T. eques* observed in the field. In (b.), *Ephedra* is listed separately because it is an aphyllous shrub with photosynthetic stems and does not satisfactorilly fit into any of the other categories.

a.) Plant family	% Time feed	% No feeds	Fed on: No genera	Fed on: No spp	Contacted: No genera	Contacted: No spp
Aizoaceae	0.36	0.37	1	1	1	1
Amaranthaceae	0.01	0.12	1	1	3	3
Asteraceae	16.28	10.67	5	5	6	6
Cactaceae	0.32	0.50	1	1	1	1
Chenopodiaceae	2.14	1.36	1	1	1	1
Ephedraceae	5.00	2.61	1	1	1	1
Euphorbiaceae	12.92	8.56	3	6	3	6
Fabaceae	15.76	18.11	3	3	3	3
Malvaceae	0.42	0.99	4	5	4	5
Molluginaceae	3.18	2.48	1	1	1	1
Nyctaginaceae	0.88	2.61	2	3	2	3
Rubiaceae	0.91	0.37	1	1	1	1
Poaceae	10.48	13.52	6	9	11	16
Polygonaceae	0.63	0.62	1	1	1	1
Portulacaceae	17.24	21.59	2	3	2	3
Solanaceae	0.50	0.50	1	1	1	1
Debris	5.74	7.07				
Unidentified	7.23	7.94				

b.) Food type	% Time feed	% No feeds
Woody seedling	0.29	0.99
Woody leaves	9.32	10.48
Woody flowers	18.18	11.60
Woody bark	1.65	1.74
Herb leaves	43.14	44.91
Herb flowers	0.96	0.87
Grass leaves	4.96	7.94
Grass flowers	4.51	4.84
Cactus cladode	0.32	0.50
Ephedra	4.96	2.61
Organic debris	11.72	13.52

noteworthy that grasses dominated both in species abundance and total ground cover, yet these were fed upon relatively rarely. Table 6b shows that there were more species of grass contacted than any other family (16); this is almost certainly an underestimate since the table shows only species that were identified positively and grasses were represented disproportionately among the unknowns.

Insects frequently bit, and were sometimes recorded taking extended "feeds", on apparently non nutritional material (e.g., see insect no. 8 in Fig. 5). Items listed as inorganic items in Table 5 include plastic marker flags, rubber on shoes and a nylon camera bag. In some instances, as mentioned in the Methods section, this chewing was not accompanied by ingestion. Moreover, *T. eques* was frequently observed taking extended feeds on a polystyrene cooler box, although none of the observation insects encountered this so no such feeds were recorded. It is possible that the insects were actually ingesting this, as evidenced by the damage to the surface of the cooler box.

b. *Dietary mixing*

Individual polyphagy was a notable aspect of the behaviour of this species. Individuals ate between 1 and 30 different food items per day, with median 11. Excluding insect no. 13 which was disturbed during the morning foraging period, the range was between 6 and 30 with median 11.5. Even within

meals, an individual generally ingested a variety of plant species/parts, and examples of this behaviour can be seen in Fig 5. The number of different food items eaten in individual meals ranged between 1 and 11 (see Fig. 4).

There were strong suggestions that dietary breadth was related to time spent locomoting by insects observed over a full day period. The time spent locomoting was positively correlated with the total number of potential dietary items rejected ($r=0.481$, $p=0.048$), the number of different dietary items rejected ($r=0.539$, $p=0.044$) the total number of feeds ($r=0.586$, $p=0.018$), and the number of different items fed on ($r=0.559$, $p=0.037$). The average feed size was negatively correlated with the time spent locomoting ($r=-0.485$, $p=0.032$).

One possible manifestation of the need to mix diets is a declining acceptability of any one food such that a different one will become relatively more acceptable over time. To examine this the data were analyzed in two ways (Table 7). Firstly, feeding bouts on the same plant species over the whole day were listed for each individual. If, for example, the second feeding bout on a given species was shorter than the first a minus was scored, and if bout 3 was shorter than bout 2 another minus was scored. If a succeeding bout was longer than the previous feed on the same plant species, a plus was scored. In this way, each plant on which a given individual had more than one feeding

bout was assigned a plus or a minus. Each insect was then scored as a plus (indicating, overall, a trend towards increasing feeding bouts) or a minus (decreasing duration of feeding bouts) depending on whether plus- or minus-scored plants predominated. These data provide an overall picture of whether successive feeds on the same plant tended to decline, even if there were intervening feeds on a different plant.

The second approach to this question was an examination of the size of successive feeds when insects switched between plant species. Feeds on new plant species were compared with the feed immediately preceding it and scored as minus or plus depending on whether it was smaller or greater. Each insect was then scored as overall plus or minus depending on which predominated.

Table 7 shows a comparison of successive feed sizes on the same plant species and when the insects switched between species. The duration of successive feeding events on the same species tended to decline, suggesting that plants became less palatable with time spent feeding on them. There was, by contrast, a tendency for feed duration to increase following a switch between foodplants. The latter provides an important control. A-priori, it might be expected that feed size would decline with time, since an insect is usually more replete on a subsequent than a previous feed. However, since feed duration tended to increase with

Table 7. Measures of whether successive feeds on the same plant species or different plant species are smaller or larger. Probabilities of non-random numbers based on sign tests of overall sign (+ or -) for 23 (same-plant data) or 26 (new-plant data) different insects.

	Successive feeds on same plant	Successive feeds on different plant
Number of insects	23	26
Average number of data points/insect (± SE)	10 ± 2	4 ± 1
Median " " " " "	6	3
Number of insects with an average of decreasing meal size	17	4
Numbers of insects with an average of increasing meal size	3	20
P <	0.005	0.01

switches between species, this suggests that declining feeds within species was not a function of time alone.

To quantify the degree of switching between food items, a switching ratio was calculated for all individuals that were observed to feed on two or more foods (Table 8). This is the total number of switches between foods divided by the number of feeding bouts. Thus, two successive bouts on one food followed by two successive bouts on another would give a switching ratio of 0.25. Table 8 shows that switching levels were very similar among individuals, whether periods of observation were all day or only part of the day. Switching ratios were also computed separately for morning and afternoon foraging periods of those full day insects that fed during both periods. A comparison of these revealed that insects tended to switch more between food items in the afternoon than in the morning feeding periods (Table 8).

Table 8. Levels of food switching between feeding bouts, expressed as number of switches between foods/number feeding bouts. Insects for which comments were lost and those that fed on only one food item are excluded. For insects observed on overcast days, morning and afternoon foraging periods are delineated by the midpoint of their feeding day.

Mean (s.d.) for all full-day insects (n=10)	0.41 ± 0.12
Mean (s.d.) for all part-day insects (n=17)	0.38 ± 0.22
Morning period for full-day insects (n=8)	0.36 ± 0.98*
Afternoon period for full-day insects	0.53 ± 0.12

Morning and afternoon significantly different with paired t-test ($t = 3.2$, 2-tailed $p < 0.014$)

DISCUSSION

This study is the first to quantify detailed aspects of patterns of feeding and dietary selection by individual herbivorous insects in the field. It has proved particularly rewarding to examine an acridid, since it is among acridids that feeding patterns and control mechanisms have been studied most extensively in the laboratory (Simpson 1990). In addition, the Acridoidea constitutes a taxon dominated by polyphagous species (Chapman 1982) and there has been almost no information on dietary mixing by individuals in the field, or the degree to which individuals are polyphagous relative to the species. Apart from a study on *Dociostaurus* by Ben Halima et al. (1985), previous field studies of dietary selection in acridids have been at the population level (see Chapman 1990 for review), and this has obvious limitations compared to the study of individual insects (e.g., Wellington 1977, Fox & Morrow 1981). This lack of field data for individuals is partly due to the great difficulty of observing them for long periods in their natural environment.

Time budgets

Overall, the amount of time spent feeding averaged 6.9%, and did not differ between insects observed on overcast and sunny days. Previous studies on acridids have recorded comparable figures: Chapman & Beerling (1990) found that

first instar nymphs of *Schistocerca americana* in the laboratory spent approximately 5% of the daylight hours feeding, while Joern et al. 1986 record that the grasshoppers *Amphitornus coloradus*, *Mermiria bivittata* and *Melanoplus confusus* fed for 5%, 10% and 4% of the time, respectively. Blaney et al. 1973 recorded that fifth instar *Locusta migratoria* in the laboratory spent from 10 to 17% of the time feeding, depending on the stage in the instar. Chapman (1959) found that nymphal *Nomadacris septemfaciata* spent 15-20% of the time feeding whenever the temperature was suitable. This is comparable to the percentage time spent feeding in the foraging periods of *T. eques* observed on sunny days in the present study (17.6% in the morning, and 14.8% in the afternoon - see Table 2).

These apparent similarities should not be interpreted to imply that it is the time spent feeding per se which is regulated by insects. Since feeding is regulated to a large extent by feedback information about the quality of food ingested (Barton Browne, 1975; Simpson & Bernays 1983; Simpson & Simpson 1990), the percentage time spent feeding is likely to increase due to compensatory feeding on nutrient poor foods. An extreme example of this are *Manduca sexta* caterpillars, which spent up to 80% of the time feeding on tobacco leaves, and never exceeded 25% when fed artificial diets on which they grew equally well (Reynolds et al. 1986). Time spent feeding has also been found to vary

within (Blaney et al. 1973; Simpson 1982) and between instars (Reynolds et al. 1986), and to be strongly related to temperature (Blaney et al. 1973) and insect weight (Reynolds et al. 1986; Bowdan 1988). Indeed, fifth instar *M. sexta* under controlled laboratory conditions and all fed discs of tomato leaves (a preferred food) ranged in time spent chewing between 0.9 and 34.8% of the daily activity (Bowdan 1988). It therefore seems that the range of 0.7 to 12.7% observed for *T. eques* in the field (Table 1) is not unduly large, considering that the insects could not be standardised beyond sex and stage in the life cycle (adults), and considering the wide range of foods taken.

Perhaps most interesting, are the similar times spent feeding by insects observed on overcast and sunny days. Insects observed on sunny days spent long periods roosting (Fig. 6), during which time thermoregulatory requirements (Whitman 1987; Chappell & Whitman 1990) prevented them from foraging. It might therefore, at first sight, be expected that insects would feed more if the temperature constraints were lifted (e.g., on overcast days). That they did not do so, may suggest that they have evolved to feed maximally under the regime in which they have an enforced extended midday roost; as discussed below, the fact that they took distinct meals separated by non-feeding periods during foraging periods on sunny days further attests to this. It might, in addition, be that feeding requirements were

reduced by lower temperatures on overcast days. This is unlikely to be the only explanation, since Raubenheimer & Bernays (unpublished) found that under laboratory conditions *T. eques* given continuous access to fava beans and wheat germ did not eat more than those deprived over the equivalent of the midday roost. On the other hand, insects observed on overcast days spent a greater proportion of the intermeal intervals locomoting, and time spent locomoting was positively correlated with dietary diversity. Therefore, insects not constrained by high midday temperatures may locomote more in order to improve dietary mixing.

The amount of time spent locomoting by *T. eques* on both sunny (14.6%) and, particularly, overcast days (27.0%) was relatively high compared with previous studies. Joern et al. (1986) found that the cryptic forb feeder, *Melanoplus confusus*, locomoted for 7.6% of the day while the cryptic grass feeders *Amphitornus coloradus* and *Mermiria bivittata* spent 2.6 and 1.3% in locomotion. These figures are appreciably lower THAN for *T. eques*, and may reflect the predator avoidance behaviour of cryptic species. Another polyphagous aposematic species, *Dactylotum variegatum*, was also found to be very active, locomoting for 14% of the day (J.Lee, unpublished). Considering the relationship observed in the present study between time spent locomoting and dietary mixing, it does not seem unreasonable to predict that polyphagous species will in general be found to be more

active than specialists.

While the proportion of time spent locomoting, feeding and quiescent by insects observed on sunny days did not differ between the morning and afternoon foraging periods, the insects foraged for significantly longer in the morning period (Table 2). According to the predictions developed for vertebrate systems, selectivity should be reduced in the shorter afternoon foraging period and also as the enforced nighttime fast approaches (Lucas 1987). This was clearly not the case however, as selectivity was actually higher in the afternoon foraging period, as evidenced by the higher rate of switching between foods (Table 8). That such predictions for vertebrates do not always hold for insects is not surprising. Being homoiothermic, vertebrates have a greater premium on obtaining energy reserves for the nighttime fast. *T. eques* may not effectively digest their food over the 12 h nighttime fast when temperatures are low, and it would be more valuable to remain selective for whatever currency is at a premium.

Patterning

In all insects examined to date, feeding events in the laboratory were not distributed uniformly or randomly in time but were clustered into bouts, or meals (Simpson 1982; Reynolds et al. 1986; Bowdan 1988; Simpson et al. 1989; Chapman & Beerling 1990). That the same may be true for

insects in the field is not altogether surprising, although *T. eques* provides a more rigorous test of this than most insects.

T. eques is, owing to thermoregulatory considerations arising from its large size and aposematic coloration (Whitman 1987), apparently constrained in the amount of time it can spend feeding. Added to this constraint is its high degree of polyphagy which appears from the present study, rather than the indiscriminate ingestion of all available plants, to be driven by selective mechanisms which facilitate dietary mixing (see below). The latter has the consequence that a proportion of the available feeding time must be devoted to searching behaviour. Given these constraints, it may seem that feeding during the morning and afternoon foraging periods would be more or less continuous, governed only by extrinsic factors such as plant distribution (i.e, that each period of foraging would itself constitute a meal). Rather, the insects took meals within the foraging periods, and usually took quiescent periods of 3 mins or more roosting on a perch between meals as has been observed for locusts in the laboratory (Simpson 1990; Chapman & Beerling 1990). This suggests that the insects are constrained by intrinsic factors, such as post-ingestive processing time, and it is the interaction of these constraints with the ecological factors which determine the patterns of feeding.

Considering the greatly enhanced complexity of the extrinsic (ecological) factors in the field, it might be expected that patterns of feeding would be considerably more regular in the laboratory. Interestingly, this appears not to be the case. Already mentioned is the observation that, like *T. eques* in the field, *L. migratoria* in the laboratory take nibbles, even on highly palatable food, which are separated from other feeding events by gaps exceeding the duration of the bout criterion for intermeal intervals (Simpson 1981, 1982, 1990). Furthermore, comparison of Figure 4 with equivalent plots for *L. migratoria* fed wheat in the laboratory (Simpson 1981, 1990) reveals no appreciable difference in the regularity of spacing or duration of meals.

Simpson (1982, 1990) suggests that it is the complex interaction of internal control mechanisms which accounts for the relative irregularity of feeding patterns under the simplified environmental conditions of the laboratory. Locusts have an short-term endogenous rhythm running at a period of about 15 mins, and the initiation of behaviours such as feeding tends to coincide with the peaks in this rhythm (Simpson 1981). However, the number of 15 min. cycles separating two feeding events is variable and, when external influences are constant, dependant on the coincidence of intrinsic events. Thus, if other physiological events that magnify the probability of initiating feeding (e.g., those

associated with defecation - Simpson & Ludlow 1986) coincide with a peak in the endogenous rhythm, the combined influence may result in feeding sooner than may otherwise be expected. It is this non-deterministic, statistical, aspect to behaviour which is thought to account for the relative irregularity of feeding patterns even in a simplified laboratory environment. On the other hand, this non-determinism imparts a flexibility on behaviour, enabling insects to respond in a variety of ways to unpredictable external stimuli (Simpson 1982). Such flexibility may "dampen" the stochastic effects of complex environments, allowing *T. eques* in the field to maintain more regular patterns of feeding than may otherwise be expected.

While the comparable regularity of feeding patterns may suggest a superficial similarity between acridids studied in the field and the laboratory, there are fundamental and important differences in the components to these patterns. Indeed, such differences exist even within species under different laboratory conditions or given different foods, and between individuals under the same conditions (Simpson 1982). In between-species comparisons, it might be expected that the components of this patterning would be set through natural selection by the ecological circumstances in which the different species evolved. It is therefore interesting that for *T. eques*, an apparently obligate generalist in an environment characterised by sparse, patchily distributed

vegetation, the degree of locomotion was the variable which seemed the most responsive to extrinsic, environmental factors. For example, both the total time locomoting (Table 1) and the proportion of time locomoting within intermeal periods (Fig. 6), increased on overcast days when the temperature constraints were reduced. While this did not lead to increased time spent feeding, increased locomotion was related to improved opportunities for dietary mixing.

Additionally, the only apparent correlations between feeding behaviour and the intermeal intervals preceding and following meals involved locomotion: larger meals tended to follow long periods of locomotion, and insects locomoted less after large meals (Table 4). Both correlations make functional sense in relation to the life style of *T. eques* and the nature of its habitat: good feeds indicate lucrative patches, and reduced locomotion following a larger meal would maximise the exploitation of that patch for subsequent meals. Chapman (1957) similarly observed that the locust *Nomadacris septemfaciata* was less active in patches of the more preferred foods, and that this lead to localised concentrations in such patches. This also makes sense in terms of the apparent necessity for *T. eques* to eat a variety of foods (further discussed below): patches dominated by previously palatable but currently rejected plants will lead to small meals, hence increased locomotion and enhanced opportunity to locate different foods.

Likewise, the necessity to locomote long distances before feeding signifies that the insects have passed through an area without suitable food and should therefore capitalise on the newly encountered patch.

These correlations contrast with those observed for insects in the laboratory. *L. migratoria* showed a positive relationship between the total duration of an intermeal interval and the size of the preceding meal (Simpson & Ludlow 1986). In contrast to *T. eques* in the field, this relationship was almost entirely due to time spent quiescent during the postprandial period, since in 90% of cases the insects fed within 1 min. of breaking quiescence. The amount eaten in a meal by *L. migratoria* was, however, not correlated with any component of the intermeal preceding it. Chapman & Beerling (1990) found that meal size was positively correlated with the duration of the preceding intermeal interval in first instar nymphs of *Schistocerca americana* and, again in contrast with *T. eques* in the field, the correlation was due to preprandial quiescence rather than locomotion. Similarly, in the blowfly, *Lucilia cuprina*, the time spent quiescent following a meal is positively related to the size of the meal but the total duration or time spent locomoting in the postprandial period are unrelated to previous meal size (Simpson et al. 1989). It therefore seems that, despite other differences between species, a common denominator among laboratory studies to

date is the relationship between feeding and quiescence and the lack of correlations involving locomotion. An interesting question is whether the contrast with *T. eques* in the field is a species difference or a result of the different observation environments. It could, of course, be due to both: *T. eques* may be capable of adjusting locomotion or quiescence to the parameters of feeding depending on the perceived characteristics of the environment.

Dietary breadth

My data indicate that in the wild, individuals of *T. eques* are remarkably polyphagous, and that the major dietary item is leaf tissue of annual herbs. This confirms the data of Whitman & Orsak (1985), who found in no-choice tests that caged *T. eques* fed on a wide variety of native shrubs and herbs. Indeed, in the present study dietary breadth was almost certainly underestimated since a large proportion of foods are ephemeral, so that the available food plants change with season (Whitman & Orsak 1985). A notable difference between the results of the present study and that of Whitman & Orsak (1985), is that the leaves of *Acacia constricta* were avoided by caged animals but formed a part of the diet in the wild (Table 5). This may reflect feeding history, since caged animals were fed oats and vegetables for 3 h, then deprived for 8 h prior to the test. It may also reflect chemical changes in the leaves, which were

picked prior to presentation to the caged animals.

The degree of dietary mixing by individuals was extremely high, with different food items being ingested in different meals as well as within individual meals (Figs 4 and 5). What mediates this behaviour? The subjective impression is that few food items are fully satisfactory. Insects usually stopped feeding on an item before it was finished and before they had fed to repletion, as evidenced by their going on to feed on another item shortly thereafter. One possibility is that most plants in the habitat of *T. eques* contain feeding deterrents, and a high proportion of the diet of this species is obtained by taking lots of small feeds on marginally acceptable plants. It may also be that dietary mixing in *T. eques* is facilitated by a coarse behaviour rule (Bookstaber & Langsam 1985), according to which feeding on any one item stops after a relatively short period regardless of the quality of the item. This is supported by the remarkable similarity in the degree of switching between individual insects (Table 8). If insects were responding to the quality of food per se, a greater difference in switching rates might be expected between individuals since insects which chanced upon high quality plants would switch less than those which encountered poorer patches.

There was, nonetheless, variability in the acceptability of individual plant species as measured by feeding bout length (Table 5). This would suggest that any decision rule

involves the monitoring of characteristics of the different dietary items using the sorts of mechanisms established for other acridid species, but with thresholds for stopping feeding on any one item set at a generally low level. Several mechanisms known to operate in feeding by acridids could be involved.

It is probable that at some level the insects are monitoring post-ingestively the quality of food. Such feedback about the nature of food ingested could have direct effects on feeding, or could be paired with other information in associative learning (Papaj & Prokopy 1989). An example of direct effects has been established for *L. migratoria*, in which high levels of amino acids in the haemolymph of well fed insects reduces the sensitivity of the palp chemoreceptors to protein, resulting in a raised threshold for the initiation of feeding (Simpson & Abisgold 1985; Abisgold & Simpson 1987, 1988).

The role of learning in ecological contexts has had much recent attention (Papaj & Prokopy 1989), although the role of learning in grasshopper foraging behaviour has remained a subject for laboratory studies. In the present study, there were many examples of short-term declining acceptability of plant species, as shown in Table 7. Although there are other possible explanations (see below), this is consistent with the use of aversion learning as a behavioural mechanism facilitating dietary selection, as has

been demonstrated by previous laboratory data for the grasshopper *Schistocerca americana*. Lee & Bernays (1988, 1990) found that these insects learned to avoid moderately acceptable foods when a meal was followed by an injection of one of a variety of plant toxins. Aversion learning based on the post-ingestive monitoring of dietary nutrient content has also been suggested for *S. americana*, both in relation to the absence of suitable dietary sterols (Champagne & Bernays in press) and protein deficiency (Chapter 3).

However, the ability to pair feedback information with specific foods may be hindered in instances, such as was observed in the present study, where several foods are ingested over a short time period (Rozin 1976; Zahorik & Houpt 1981). An interesting pattern in the present data may to some extent answer this. On a total of 13 occasions, individuals took a sequence of meals on a single species, interrupted by contacts and rejections of different species. This would not be surprising in cases where the sequence food is much higher on the acceptability hierarchy than the alternatives. However, in seven of the cases the alternative food was a much more generally acceptable species than the sequence food, and yet was rejected during the sequence. Three such examples were as follows (see Table 5 for acceptability ranking; the numbers in parenthesis refer to the codes in the table):

Sequence food	Alternative food
1. *Bouteloua barbata* seeds (18)	*Allionia incarnata* (3)
2. *Portulaca oleraceae* (19)	*Acourtia nana* (16)
3. *Prosopis juliflora* (35)	*Solanum eleagnifolium* (21)

In each case, the total time period involved was less than 10 minutes. This may suggest a very short-term specialization which would be consistent with the need to monitor the chemical nature of novel foods.

An additional possible explanation for the short-term declining acceptability of plant species observed in the present study (Table 7) is 'sensory specific satiety' observed in mammals (Rolls et al. 1983; Le Magnen 1985; Rolls 1989). In rats, monkeys and humans, flavours become progressively less acceptable in proportion to the time spent feeding on them, while other flavours may remain attractive. This has been interpreted to suggest that satiation is partially a result of waning pre-ingestive, sensory cues with prolonged experience of a specific flavour (e.g., Kraly et al. 1978). However, as has been argued by Le Magnen (1985), this sensory satiety may itself be a conditioned response which is reinforced by associative pairing with post-ingestive feedbacks. It is therefore not at all clear whether, at the level of mechanisms, sensory specific satiety can be distinguished from aversive associative learning. Perhaps they are distinct only in

occuring at different areas of the same functional spectrum: aversion learning may relate to longer-term, adaptive aversions and sensory specific satiety to short-term feedbacks facilitating nutritional balance through dietary mixing.

The related phenomenon, neophilia, may also occur, as has been suggested for *S. americana* in the laboratory (Chapter 3). The fact that feed durations increased when insects switched to feeding on new plants (Table 7) is consistent with this. Also consistent with this, is the curious phenomenon of biting on inert materials. The insects may initiate biting on any novel item that is not initially deterrent, then continue feeding for long enough to monitor feedbacks (see also Blaney et al. 1985, p. 39, for a similar interpretation).

What are the possible advantages for *T. eques* of the observed high level of individual polyphagy? Most likely, is the ability to obtain a nutritionally balance diet. If various plants contain nutrients in proportions which are skewed relative to the insects' dietary requirements (e.g., Chapter 2), then the too frequent ingestion of any one plant will result in an imbalanced diet. As has been suggested by Parker (1984) for the grasshopper *Hesperotettix viridis*, frequent switching between foods would by chance alone provide an opportunity to correct such imbalances. Additionally, the mechanisms discussed above would enable

the animals to respond more selectively to foods, and thus meet at a more fine-grained level its specific nutrient requirements.

Dietary mixing could, in addition, relate to plant secondary chemistry. Freeland & Janzen (1974) have suggested that polyphagy is a means of spreading the toxic load of various potentially deleterious allelochemicals ingested, although no mechanisms for how this is accomplished were mentioned. The observed decline in meal size by *T. eques* on successive within-species encounters with foodplants could be explained in this way, with the acceptability of specific plants declining in proportion to the levels of particular allelochemicals ingested. It might also be, as has been suggested for the related *Romalea guttata*, that the need to sequester a variety of plant toxins drives its feeding on a diversity of plants (Jones et al. 1987). This would be consistent with the preference for the leaves of seasonal herbaceous plants (Table 6b) which generally contain high levels of secondary compounds, relative to the low acceptance of grass leaves which generally do not.

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Chapter 5

Analysis of covariance:
an alternative to nutritional indices

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Abstract. Some statistical problems are added to the growing list of cautionary tales regarding the use of the conventional, ratio-based nutritional indices (RCR, RGR, ECI, AD and ECD). Analysis of ratios is based on the, probably unrealistic, assumption of an isometric relationship between denominator and numerator variables. Analysis of covariance (ANCOVA) makes less restrictive assumptions, and additionally provides important information about the data which is lost in the use of ratio variables. Here I demonstrate, using artificial data sets, some of the pitfalls of statistical analysis of ratios and illustrate how these may be avoided using ANCOVA. Some possible consequences of such statistical iniquities for biological interpretations are discussed.

Introduction

In his classic paper of 1968, Waldbauer proposed a set of gravimetrically determined indices, based on ratios and rates, for use in the quantitative study of insect nutrition. Relative consumption rate (RCR) and relative growth rate (RGR) are the amounts eaten and the weight increment of insects, respectively, standardised for the experimental period and some measure of the mass of the insect. Approximate digestibility (AD) is the difference between the amount of food eaten and that egested in the

frass, standardised for the amount of food eaten. Efficiency of conversion of ingested food (ECI) and assimilated food (ECD) are the mass increment of the insects per unit of food eaten and absorbed, respectively.

These indices have been widely adopted in ecological, physiological and behavioural studies, to the extent that they now underlie much of the published data on quantitative aspects of insect nutrition and nutritional ecology. However, the indiscriminate use of these indices has recently been criticised for methodological reasons. Schmidt and Reese (1986) demonstrated, using an artificial data set, how errors could accumulate and amplify in the calculation of the standard utilisation indices, leading to inaccurate and often erroneous conclusions. Van Loon (1988a, b, in press) used a flow-through respirometer as a methodological check on the accuracy of energy budgets determined for two *Pieris* species using the standard gravimetric methods. There were serious discrepancies between the two methods, which applied to both the effect of dietary treatments relative to control groups and to the absolute values of control groups. Van Loon (1988a) concluded that "..the reliability of results using the gravimetric method in the majority of published reports must be critically reconsidered". There are also problems involved in the biological interpretation of the indices which relate to their physiological bases. For example, AD is influenced by the proportion of non-

nutritive substances in the food (Simpson and Simpson, 1990 pp 138-139) and by secretory products such as peritrophic membrane (Bernays and Simpson, 1990).

In this paper I consider some additional, statistical, problems that might arise in the use of the standard nutritional indices. These centre around the use of ratios to increase precision of measurements or to remove the effects of confounding variables such as body size. As an alternative, I recommend analysis of covariance (ANCOVA) (Fisher, 1932; Cochran, 1957). ANCOVA has several advantages over the use of ratios in the analysis of biological data, including: more powerful tests of hypotheses (i.e. decreased type II error rate); a more detailed analysis (i.e., provision of more information about the data set); reduced incidence of artifactual treatment effects; a greater reduction in the error around the dependent variable; and in certain cases, greater robustness than the analysis of ratio variables. I use artificial data sets to illustrate some of the problems that might arise in the use of ratio variables, and demonstrate how these can be avoided using ANCOVA.

Methods and results

A single data set, divided into two hypothetical treatments of fifty observations each, was used as a basis for the

independent (X) variable (denominator in ratios) for most analyses (henceforth referred to as the baseline data). These data, generated using a pseudorandom number generator (SPSS/PC+), were normally distributed and had means and variances representative of dry weights (mg) of female *Locusta migratoria* at the start of the fifth instar. The means $\pm$ variances were as follows: treatment 1 = 150.2 $\pm$ 37.3, and treatment 2 = 149.9 $\pm$ 34.5. In cases where the X variable was made to differ between treatments, a constant was added or subtracted from all values of the baseline data in one of the treatments. Dependent (Y) variables (numerator in ratios) with stipulated relationship to the X variable were generated by performing addition, subtraction, multiplication or exponentiation on the X variable. An error term was incorporated by adding to each datum point in the Y variable, at random, a number from a normally distributed set of pseudorandom numbers with a mean of approximately 0 and stipulated variance 25. In all cases, ratio variables were normally distributed with statistically equal variances and therefore required no transformation.

In what follows I illustrate some of the problems which can arise when ratios are used to standardise one variable (numerator) for the effects of another (denominator).

1) Allometric relationship.

The use of ratios to standardise one variable for the effect

of another (e.g., where initial insect weight is the denominator) assumes that numerator and denominator are isometrically related i.e., that they are related linearly and the function passes through the origin (Packard and Boardman, 1987; 1988). In other words, isometry breaks down when there is a significant non-zero y-intercept (e.g., see Fig. 1a) or when the curve describing the relationship between denominator and numerator is non-linear (Fig. 1b).

a) *Non-zero constant term:* To illustrate the effects on the analysis of ratios of a non-zero y-intercept in the plot of numerator on denominator, a Y variable was generated by adding an error term to the baseline data. This gave two treatments which did not differ statistically and which were linearly related to the X variable with regression coefficient (slope) of approximately 1. A constant value of 3 was then added to each datum point in the Y variable for one of the treatments, introducing a small but statistically significant difference between the treatments. Table 1a shows that a significant difference ($p<0.002$) between the treatments was detected using both ANCOVA on the original data and analysis of variance (ANOVA) on the ratio variable. A progressively increasing constant term was then added to both treatments, to increase the value of b in the equation $Y = aX + b$ (Table 1). A non-zero constant term is schematically depicted in Fig. 1a.

It can be seen from Table 1a that the f-ratio derived

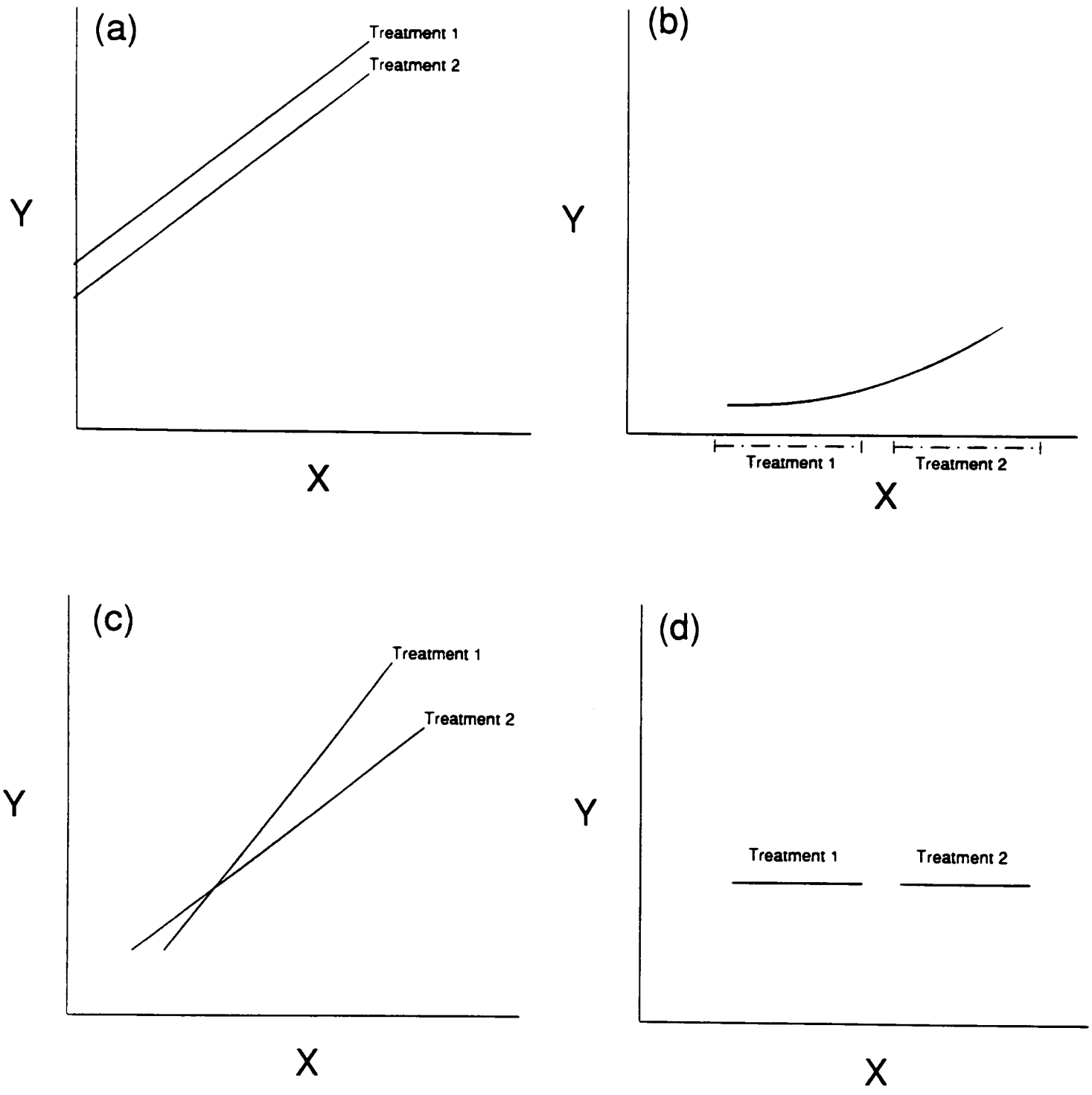


Figure 1. Some instances in which the analysis of ratio variables is statistically inadequate. a) Comparison of treatments which have parallel slopes in the regression of Y on X but differ with respect to their y-intercepts. As the distance between the origin and the y-intercept of both slopes increases, the ability of ANOVA on the ratio Y/X to detect the difference between treatments diminishes. b) Comparison of treatments which are unbalanced with respect to the X variable, when the regression of Y on X is non-linear. ANOVA on the ratio variable Y/X fails to remove the effect of the X variable on Y, and may produce spuriously significant differences between treatments. c) Comparison of treatments which differ in the regression slope of Y on X. Ratios fail to detect this multiplicative effect of X on Y and can lead to the mistaken conclusion that the treatments differ with respect to the Y variable by a constant value. d) Comparison of treatments which are unbalanced with respect to the X variable, when there is no correlation between X and Y. The use of ratios to "correct" the Y variable for the effects of X can lead to a spurious statistically significant difference between treatments.

Table 1. a) Effects of increasing non-zero y-intercepts when the ratio Y/X is compared across two levels of a hypothetical treatment. b) Regressions of the ratio variables for both treatments on the denominator variable. "Constant" refers to a constant added to both levels of the treatment.

(a)

Constant		F-ratio	Sign. of F
0	ANOVA on ratios	10.56	0.002**
	ANCOVA	10.50	0.002**
100	ANOVA on ratios	7.26	0.008**
	ANCOVA	10.50	0.002**
200	ANOVA on ratios	3.70	0.057
	ANCOVA	10.50	0.002**
300	ANOVA on ratios	2.09	0.150
	ANCOVA	10.50	0.002**

(b)

Constant	y-intercept	Slope	Correlation coefficient	Sign. of f
0	1.01	-2×10^{-5}	-0.003	0.974
100	2.36	- 0.005	-0.588	<0.001***
200	3.70	- 0.009	-0.824	<0.001***
300	5.05	- 0.014	-0.910	<0.001***

from ANOVA of the ratio variable decreased as the constant term in the regression increased. The increasing constant term had no effect on the ANCOVA across the same range of values for b. This demonstrates that the power, or the ability to detect small differences between treatments, may diminish with the use of ratio variables.

An important reason that ratio variables rather than the raw data are analyzed, is to remove the effect of the denominator variable on the numerator. If ratios are successful in this respect, there should be no correlation between the denominator and the ratio variables. Table 1b shows an increasing correlation between the ratio and denominator with an increasing value of b in the equation $Y = aX + b$ (i.e., with an increase in allometry), showing a progressive failure to remove the effect of the denominator. Similar examples are provided by Atcheley et al. (1976), Prothero (1986) and Packard and Boardman (1987).

b) *Non-linear relationship:* To illustrate some effects on the analysis of ratio variables of a non-linear relationship between denominator and numerator, a constant term of 25 was subtracted from the baseline data for one treatment. This simulated an "unbalanced" experiment, in which the average covariate value differed between treatments (e.g., different weights of males and females). A Y variable, with linear relationship to the X variable,

was then generated by adding an error term to all values of the X variable as described above. At this stage the relationship between X and Y variables was still isometric and both the ANOVA on the ratio variable and ANCOVA showed that, taking into account the effect of unbalanced treatments over the X variable, the Y variable did not differ over the two treatments (Table 2).

A Y variable (Y^{exp}) which was non-linearly related to the X variable was then generated by raising the X variable to powers from 1.02 to 1.10 before adding the error term (Table 2; Fig. 1b). The f-ratio for the analysis of the ratio variable (Y^{exp}/X) increased with an increase in the power function relating Y to X (Table 2), resulting in a spurious significant effect ($p<0.03$) at a power of 1.08.

There was, however, no such spurious effect when ANCOVA was performed with Y^{exp} as dependent variable and X as covariate, demonstrating that this procedure was more robust to non-linearity than was ANOVA on the ratio variable (Table 2). In this example, the degree of exponentiation in the function relating Y and X was relatively slight (maximum of $Y=X^{1.1}$). However, where the exponent relating Y to X is more extreme (e.g., $Y=X^2$), ANCOVA using X as a covariate may lead to inaccurate results. The best procedure is therefore to determine the function of X which best describes Y, and use this as the covariate in ANCOVA. In the present example this function was known, and Table 2 shows the results of ANCOVA

Table 2. Effects of increasing non-linearity between denominator and numerator when ratio variables are compared across two treatments using ANOVA. An exponent of 1.0 denotes a linear relationship.

Exponent		F-ratio	Sign. of F
1.00	ANOVA on ratios	0.22	0.624
	ANCOVA	0.05	0.817
1.02	ANOVA on ratios	<0.01	0.970
	ANCOVA[1]	0.05	0.818
	ANCOVA[2]	0.05	0.819
1.04	ANOVA on ratios	0.42	0.521
	ANCOVA[1]	0.05	0.819
	ANCOVA[2]	0.05	0.821
1.06	ANOVA on ratios	1.87	0.175
	ANCOVA[1]	0.05	0.821
	ANCOVA[2]	0.05	0.823
1.08	ANOVA on ratios	4.91	0.029*
	ANCOVA[1]	0.05	0.822
	ANCOVA[2]	0.05	0.824
1.10	ANOVA on ratios	10.24	0.002**
	ANCOVA[1]	0.05	0.824
	ANCOVA[2]	0.05	0.826

1: Linear function of X used as a covariate
2: Best-fitting exponential function of X used as covariate

using both the linear and correct exponents as covariates. Where the exponent is unknown, the procedure is first to plot the data to obtain a visual estimate of the relationship. Then use stepwise multiple regression with Y as dependent variable and the X variable raised to a range of estimated powers entered sequentially as independent variables. The exponential function which explains the most variation around the Y variable is used as a covariate in the ANCOVA. Alternatively, a least-squares estimate of the best fitting equation could be obtained using an appropriate algorithm available on statistical packages for microcomputers.

2) Non-parallel slopes

A prerequisite for comparing adjusted means using ANCOVA is that the slopes describing the relationship between covariate and dependent variable are parallel, and a properly designed ANCOVA therefore incorporates a term testing for a treatment x covariate interaction. A significant treatment x covariate interaction (depicted in Fig. 1c) indicates that the covariate has a different effect on the dependent variable over the two treatments. In this instance a comparison of treatment means does not make sense since any difference may fall away, become larger, smaller or even reversed depending on the range of covariate values covered in an experiment (Fig. 1c). In some instances, the

full range of possible covariate values may be represented in an experiment. In this case it might be of interest to determine whether the values in one treatment are consistently greater than those in the other (i.e., whether there is no point throughout the range of X values where the regression lines meet or cross). Both parametric (Tsutakawa and Hewett, 1978) and nonparametric (Tsutakawa and Hewett, 1977) tests are available for this.

The analysis of ratio variables does not take into account such interactive effects between denominator and treatments. This can result in the loss of important information and lead to incorrect conclusions about treatment effects. To illustrate, a Y variable was generated from the baseline data by multiplying all values in one treatment by 1.3, then adding the error term to both this and the unaltered values of the second treatment. This gave two treatments with slopes of approximately 1 and 1.3.

The data were analyzed using both ANOVA on the ratio variable and ANCOVA. The ANCOVA model included a term for a treatment x covariate interaction; this was significant, indicating that the effect of X on Y was different across the two treatments (Table 3a). The final design (Table 3b) therefore fitted separate regression models within each level of the treatment.

By contrast, analysis of the ratio variable Y/X suggested that, having "removed" the effect of X on Y, the mean values

Table 3. Comparison of ANCOVA on raw data (a and b) and ANOVA on the dependent variable/covariate ratio (c) where the regression coefficient of Y on X differs across two hypothetical treatments.

(a)

Source of Variation	SS	DF	MS	F	Sign. of F
ERROR	2839.43	96	29.58		
COVARIATE	4691.92	1	4691.92	158.63	.000
TREATMENT	16.23	1	16.23	.55	.461
COV. BY TREAT.	169.64	1	169.64	5.74	.019

(b)

Source of Variation	SS	DF	MS	F	Sign. of F
ERROR	2839.43	96	29.58		
TREATMENT	16.23	1	16.23	.55	.461
COV. WITHIN TREAT.	4797.45	2	2398.73	81.10	.000

(c)

Source of Variation	SS	DF	MS	F	Sign. of F
ERROR	0.13	98	.00		
TREATMENT	2.30	1	2.30	1793.39	.000

for the treatments were different (Table 3c). Therefore, the analysis of a ratio variable lead to both the loss of the important information that the effect of the covariate differed between treatments, and the potentially incorrect conclusion that there was a consistent difference in the Y variable across treatments.

3) <u>No covariance between denominator and numerator</u>

The use of ratios to standardise one variable for the effect of another assumes not only the nature of the relationship between the X and Y variables (see above), but indeed that such a relationship exists. Where there is no relationship (e.g, Fig. 1d), the use of ratios poses major problems ranging from decreased power of the test (due to increased variation - e.g., see Packard and Boardman, 1987; 1988) to artifactual treatment effects.

To illustrate, a Y variable was produced by adding an error term to the baseline data as described above. A normally distributed X variable which was random with respect to the Y variable ($r = 0.02$, $p > 0.80$) was generated using a pseudorandom number generator (mean $\pm$ var for the treatments were 149.7 ± 33.05 and 155.48 ± 31.9). By adding a constant term to the X variable for one treatment only, I simulated a situation in which the "covariate" differed across levels of the treatment and there was no correlation between X and Y variables (Fig. 1d).

Table 4 shows that as the mean levels of the X variable diverged between treatments, the f-ratio of the ANOVA of the

Table 4. Comparison of ANCOVA on raw data and ANOVA on the dependent variable/covariate ratio where there is no significant covariance. Two hypothetical treatments are compared. a) ANCOVA reveals no significant covariance, therefore b) ANOVA is performed on the untransformed data. c) F-ratios and significance levels for ANOVA on the ratio variables with increasing difference in covariate values between the treatments. "Constant" refers to a constant value added to the covariate values for one treatment.

(a)

Source of Variation	SS	DF	MS	F	Sign. of F
ERROR	6386.06	97	65.84		
COVARIATE	9.41	1	9.41	.14	.706
TREATMENT	8.18	1	8.18	.12	.725

(b)

Source of Variation	SS	DF	MS	F	Sign. of F
ERROR	6395.47	98	65.26		
TREATMENT	2.72	1	2.72	.04	.839

(c)

Constant		F-ratio	Sign. of F
2.0	ANOVA on ratios	1.51	0.222
3.0	ANOVA on ratios	2.98	0.088
4.0	ANOVA on ratios	4.93	0.029*
5.0	ANOVA on ratios	7.36	0.008**

ratios increased to produce a spurious statistical significance.

By contrast, ANCOVA provides a statistical measure of the strength of any relationship between the Y variable and covariate. Where there is no relationship (i.e., a non-significant regression term; Table 4a) the covariate may be excluded from the analysis (Table 4b). Note that there was no artifactual effect even when the covariate term was included in the analysis (Table 4a); this demonstrates that, as in the case of nonlinearity, ANCOVA is more robust than ratios in this instance.

Discussion

I have demonstrated some instances in which the use of ratio variables to scale data can lead to incorrect conclusions. This is by no means the first such demonstration: the indiscriminate use of ratios has been severely criticised in several fields including evolutionary biology (Gould, 1966; LaBarbera, 1989), morphometrics (Prothero 1986), systematics (Atcheley et al., 1976; Atcheley and Anderson, 1978; Albrecht, 1978), physiology (Packard and Boardman, 1987; 1988), fisheries research (DeVlaming et al., 1982) and plant ecology (Weller, 1987). Indeed, so much so that over a decade ago Dodson (1978) was led to state: "That ratios do not remove the effect of size from the data set is so

widely appreciated as to deserve no further discussion". Not so in insect nutrition; RCR and RGR are both attempts to remove the effect of body size from the variables of interest and these, together with AD, ECI and ECD, remain firmly entrenched in the entomological literature.

A major problem with the use of inadequate statistics is the possibility that they lead to inaccurate or incorrect biological conclusions. For example, Atcheley et al. (1976) have demonstrated how spurious correlations may arise between ratios and the variables from which they are derived. One such instance is between X and Y, when X=a/b and Y=c/b (see also Atcheley and Anderson, 1978); this is reminiscent of RGR and RCR which have the product of insect weight and experimental period as a common denominator. Therefore, procedures which draw biological conclusions from the relationship between RCR and RGR should be avoided. One such procedure is that recommended by Blau et al. (1978) to distinguish toxic from deterrent effects of plant allelochemicals on herbivorous insects.

A further example is the spurious negative correlation that might arise between a ratio variable and its denominator (Atcheley et al., 1976; Prothero 1976). It has commonly been observed that consumption correlates negatively with AD (Richman, 1958; Scriber and Slansky, 1981; Jindra and Sehnal, 1989; Karowe and Martin, 1989) and this leads to the possible interpretation that animals may

compensate for low nutrient intake by increasing digestive efficiency (Scriber and Slansky, 1981; Slansky and Scriber, 1985; Simpson and Simpson, 1990 - further discussed in Chapter 2). Similarly, the ubiquitous observation that ECD correlates negatively with AD has been given various biological interpretations (Welch, 1968; Mukerji and Guppy, 1970; Kogan and Cope, 1974; Reese and Beck, 1978; Scriber and Slansky, 1981; Jindra and Sehnal, 1989; Karowe, 1989). Since amount eaten is the denominator in AD, and ECD is growth standardised for amount absorbed, the biological validity of these observations must be called into question. Reanalysis of the untransformed data using ANCOVA will test the verity of these correlations.

An important caveat in the use of ANCOVA is that there should be no treatment effect on the covariate (Cochran, 1957; Smith, 1957). It is therefore important that the measure of insect weight used should, where possible, be the initial insect weight at the start of experiments; a similar conclusion was reached by Farrar et al. (1989) with regard to the weight component in the denominator of RGR and RCR. Where "average larval weight" (Waldbauer, 1968) is used as covariate (or denominator in ratios), this may itself be affected by the treatment and would thus distort the nature of the treatment effect (Smith, 1957; Cochran, 1957). Further, the use of gain in weight (i.e., final - initial weight) is in most cases not to be recommended. This is what

Cochran (1957) calls a "home made" method of adjustment to increase precision, and has the very restrictive assumption that initial weight is linearly related to final weight with regression coefficient of 1 (Cochran, 1957). For measures of growth, it is therefore preferable to use final weight as the dependent variable and initial weight the covariate in ANCOVAs. Clearly, this criticism also applies to the use of weight increment in the ratio-based index RGR.

My simulations have illustrated ways in which important information about a data set can be lost when variables are compounded into ratios. These include the loss of information about the relationship between numerator and denominator variables, as well as about any across-treatment differences in the effects of the denominator on the numerator variable (i.e., treatment x covariate interactions). To these should be added an additional problem which may arise when more than one term appears in the denominator of a ratio variable (e.g., the relative rates RCR and RGR which have experimental period and some measure of larval weight in the denominator). In this instance, different combinations of time (experimental duration) and body weight can yield the same value for the ratio variable. The outcome could be a loss of detail regarding treatment effects or, worse still, the incorrect conclusion that there is no treatment effect when both of the denominator variables are affected equally and in

opposite directions. ANCOVA provides separate regression information about the two covariates.

I do not make the claim that all inferences drawn from ratio-based nutritional indices are flawed. Beyond the baseline data in the present study which had means and variances representative of locust dry weights, my simulations used parameter values designed to demonstrate the pitfalls of analyses of ratios. There are, however, parameter values for which there may be no discrepancy between the outcome of analysis of ratios and ANCOVAs (e.g., Packard and Boardman, 1987; 1988). The important question is to what extent do the parameters of real data for insect nutrition satisfy these assumptions. This is a question which can only be answered empirically, and the steps in properly designed ANCOVAs can play a significant role in providing the information necessary to determine this. However, considering that nutritional indices are underlain by physiological processes and their relation to growth and size, it seems a-priori that the assumption of isometry will in most instances be violated (Platt and Silvert, 1981; Schmidt-Nielsen, 1984; Prothero, 1986; Heusner, 1987; LaBarbera, 1989). Packard and Boardman (1988) examined 72 data sets (although it is not indicated what the data sets represented) and found for 28% of these that ANOVAs on ratios led to qualitatively different conclusions to ANCOVAs, and in most cases ANCOVA was more effective at

removing variation in the dependent variable that was due to the covariates.

Furthermore, several authors have defended the use of ratios under specific circumstances (e.g., Corruccini (1977) - but see reply by Atcheley (1978); Hills (1978), Dodson (1978), Albrecht (1978) - but see reply by Atcheley and Anderson (1978); Tracy and Sugar (1989) Magnusson (1989) - but see reply by Packard and Boardman (1989); Prairie and Bird, (1989)); although none of these convincingly demonstrated circumstances in which ratios were superior to ANCOVA. There may also be potential problems with the use of least squares regression in ANCOVA. Specifically, least squares regression takes into account only the variation around the Y variable, assuming that there is minimal variation around the X variable (Ricker, 1973; Harvey and Mace, 1982). The regression slope relating Y to X is progressively underestimated as the variance around the X variable increases (Harvey and Mace, 1982). Since biological covariates usually have both intrinsic variability and are subject to error of measurement, reduced major axis and major axis regression have been proposed as alternatives (Ricker, 1973; Harvey and Mace, 1982). To my knowledge the robustness of ANCOVA to situations where variability around X is large has not been explored; however, Harvey and Mace (1982) present methods for calculating and testing for heterogeneity of slopes, and comparing elevations when

reduced major axis regression is used.

In conclusion, I see several reasons why ratio-based nutritional indices should be substituted by ANCOVA. A major criterion in evaluating statistical techniques is the amount and accuracy of information they provide about the data set. ANCOVA is not only free from the restrictive, and usually unrealistic assumption of isometry upon which the use of ratios is based, but provides information on the relevant parameters. In addition, therefore, to reducing the possibility of incorrect conclusions, ANCOVA provides potentially important information which may lead to new insights into biological phenomena. Thus, covariate x treatment interactions may reveal interesting multiplicative effects of an experimental treatment. At present there is no information on the relationships between, for example, nutrients assimilated and converted to growth under different intake regimes; the implicit assumption in the use of AD and ECD is a linear relationship with equal regression coefficients across all treatments. Similarly, non-zero y-intercepts may contain information of biological relevance (e.g., see Kogan and Cope, 1974). Furthermore, ANCOVA may be more robust than ANOVA under certain circumstances (e.g., where there is no covariance in experiments which are unbalanced with respect to the X variable; where the relationship of Y on X is non-linear). Given these factors it seems that, even in the unlikely event that real data

often do not violate the assumptions of ratio variables, ANCOVA may still provide a more sound basis for quantitative nutrition than the currently popular ratio-based indices.

Finally, one of the most often proposed reasons for the use of ratios in insect nutritional ecology is the fact that they are simple to calculate and convenient to analyze. To this, Albrecht (1978) aptly counters that "ratios are best avoided because their apparent simplicity ... overlies a complex of statistical and conceptual difficulties which may affect biological conclusions". In fact, with the advent of the spreadsheet and comprehensive statistical packages for microcomputers, ANCOVA is no more difficult. It also forces more careful design of experiments and promotes better statistical practices.

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GENERAL CONCLUSIONS

The preceding chapters have revealed several factors pertinent to the ability of acridids to extract from their environment a balance of nutrients which meets their physiological requirements, and some constraints on this ability. The separate Discussions have dealt with what I consider to be the interpretations of immediate relevance to the data presented in each chapter. Here I will highlight a few aspects that I consider the thesis as a whole has identified as particularly exciting areas for future research.

First, is the question of post-ingestive compensation. As discussed in Chapter 2, there is currently no satisfactory evidence that insects are actively able to regulate the efficiency with which they process ingested nutrients. There are numerous examples where processing efficiencies (AD, ECI and ECD) were found to correlate with levels of intake (Chapters 2 and 5), but there are three major problems in deciding whether these were the outcome of a true compensatory response: 1) the nutritional indices used to measure the variables are statistically, biologically and methodologically flawed in such a way that they may, inter-alia, give rise to spurious correlations (Chapter 5); 2) where consumption and post-ingestive phenomena correlate it is, without establishing the

mechanisms, not possible to ascertain the direction of causality (Scriber and Slansky, 1981; Simpson and Simpson, 1990); and 3) it might be that the frequently observed relationship between AD and consumption is the passive outcome of an upper limit to the processing capabilities of the insects i.e., that efficiencies *decrease* with high intake, rather than increase with limited intake (Chapter 2).

It therefore appears that the traditional approach to the study of post-ingestive compensation, in which the inputs (feeding regime, dietary quality) are manipulated and the outputs (growth, nutrients egested and excreted) are measured, has reached a ceiling in its usefulness. What is needed is an approach in which the intervening mechanisms are manipulated (e.g., via neural and hormonal feedback loops, haemolymph nutrient levels etc.) and the effects on output of these manipulations measured. A similar approach has been successful in elucidating mechanisms underlying compensatory feeding (Abisgold and Simpson 1987, 1988) and dietary selection behaviour (Lee and Bernays 1988, 1990).

While the phenomenon of compensatory feeding for proteins and, to a lesser extent carbohydrates (chapter 2) has been well established, it remains to be seen whether compensatory feeding for other nutrients takes place. Chapter 2 demonstrates how compensatory feeding may be affected by the interaction between two nutrients, leading to trade-offs

between surpluses and deficits of each. This situation becomes considerably more complex where several nutrients are involved, leading to interesting questions regarding the way control systems regulating intake of the various nutrients may interact.

A particularly interesting question in this regard is whether and to what extent these interactions are adaptively coordinated to change with the changing nutrient requirements of insects - the alternative being that the conflicts which arise lead to a fixed, inflexible response. For example, it was found in Chapter 2 that protein intake was regulated more strongly in the face of interference by carbohydrates than vice-versa. This is not surprising, since the fifth instar is a period of rapid somatic growth. The crucial question is whether the relative strengths of regulation for carbohydrates and proteins change for sexually mature adults, which have relatively lower requirements for proteins. At the level of dietary selection, this question has been answered. Chyb and Simpson (1990) found that *L. migratoria* in the early, somatic growth phase of adulthood self-selected a higher ratio of proteins to carbohydrates than did older adults which had stopped growing. This can be explained by changes in peripheral sensitivity leading to a relative increase in feeding on carbohydrate-rich and a decrease in feeding on protein-rich diets by the older adults. However, where both nutrients are

in the same diet in a no-choice situation, an entirely different situation is encountered by the insect.

While Chapter 2 has demonstrated some ways in which the mechanisms underlying compensatory feeding for different nutrients may interact, an area which has thus far received no attention is the way that mechanisms regulating different modes of nutritional compensation (compensatory feeding, dietary self-selection and possibly post-ingestive compensation) interact (Simpson and Simpson 1990). At which point does an insect "decide" not to increase ingestion of a foodplant low in proteins, but rather abandon it and search for a more suitable one? The field study described in Chapter 4 recorded the *outcome* of the interactions of compensatory feeding and self-selection (i.e., feeding behaviour in a natural context), but was unable to distinguish the underlying mechanisms. In laboratory studies, such interactions can only be observed in a choice test where both selection and compensatory feeding might take place. However, choice studies often do not take into account compensatory feeding or consider it a confounding variable to be experimentally controlled for (e.g., Chapter 3).

In many instances, the same mechanisms may be involved in compensatory feeding and dietary self-selection. For example, increased peripheral sensitivity due to protein deprivation (Abisgold and Simpson 1987, 1988) will make an

insect both more likely to feed on a high-protein diet in a choice test, and to feed more frequently in a no-choice test (e.g., Chapter 1). There might, however, be phenomena which are uniquely the outcome of a choice scenario i.e., which are only expressed in a choice situation. An indication of this was found in Chapter 1. Both choice and no-choice tests were conducted to assess the effects of tannic acid on feeding behaviour of *L. migratoria*. The choice and no-choice tests were directly comparable, because they were conducted over the same time period and using identical diets. While the no-choice tests failed to detect any effect of tannic acid on detailed feeding behaviour, tannic acid was clearly deterrent in the choice tests. It is not in itself surprising that the outcome of choice and no-choice tests differ (see also Chapter 3), since it might be expected that any initial deterrence would be enhanced in the presence of an alternative, more palatable diet (Cook 1976). What is surprising, is that no initial deterrence was detected in the no-choice test (this should be apparent as an increased latency before feeding and/or a decreased size of first meal). The suggestion is, therefore, that the presence of an alternative diet might actually *elicit*, rather than enhance behaviours. Further investigation of such phenomena may lead to new insights into the process of nutritional compensation and its underlying mechanisms.

In conclusion, the work described here has provided some

new insights into the subject of nutritional compensation and its constraints in acridids. These insights emphasise the great complexity of the interaction between intrinsic mechanisms and external variables, and stress the need to pay special attention to methodological aspects of research in this area. In recent years, there have been constructive developments in the laboratory techniques applied to the study of insect nutrition. The use of artificial diets combined with detailed behavioural observations has played a central role in elucidating mechanisms underlying compensatory feeding (Simpson and Simpson 1990) and dietary self-selection (Simpson et al. 1988, Chyb and Simpson 1990). In Chapters 1 and 2, this approach has been used for the first time to study the effects of allelochemicals on compensatory feeding. Such techniques have also been used successfully in the study of insect learning. Simpson and White (1990) used a combination of artificial diets and plant-produced odours to demonstrate that *L. migratoria* may develop a "learned hunger" for proteins, and in chapter 3 similar techniques using plant-produced flavours were used to investigate a novelty effect underlying dietary selection by *S. americana*. By contrast, the study described in Chapter 4 lies at the extreme opposite end of the methodological spectrum. There, feeding behaviour was recorded in the field, providing an opportunity to observe the way insects respond to the full complexity of a natural environment, but

at the price of reduced insight into the mechanisms. What is now needed are studies which bridge this gap, using the incisive tools of laboratory research to increasingly emulate the complexity of a natural environment.

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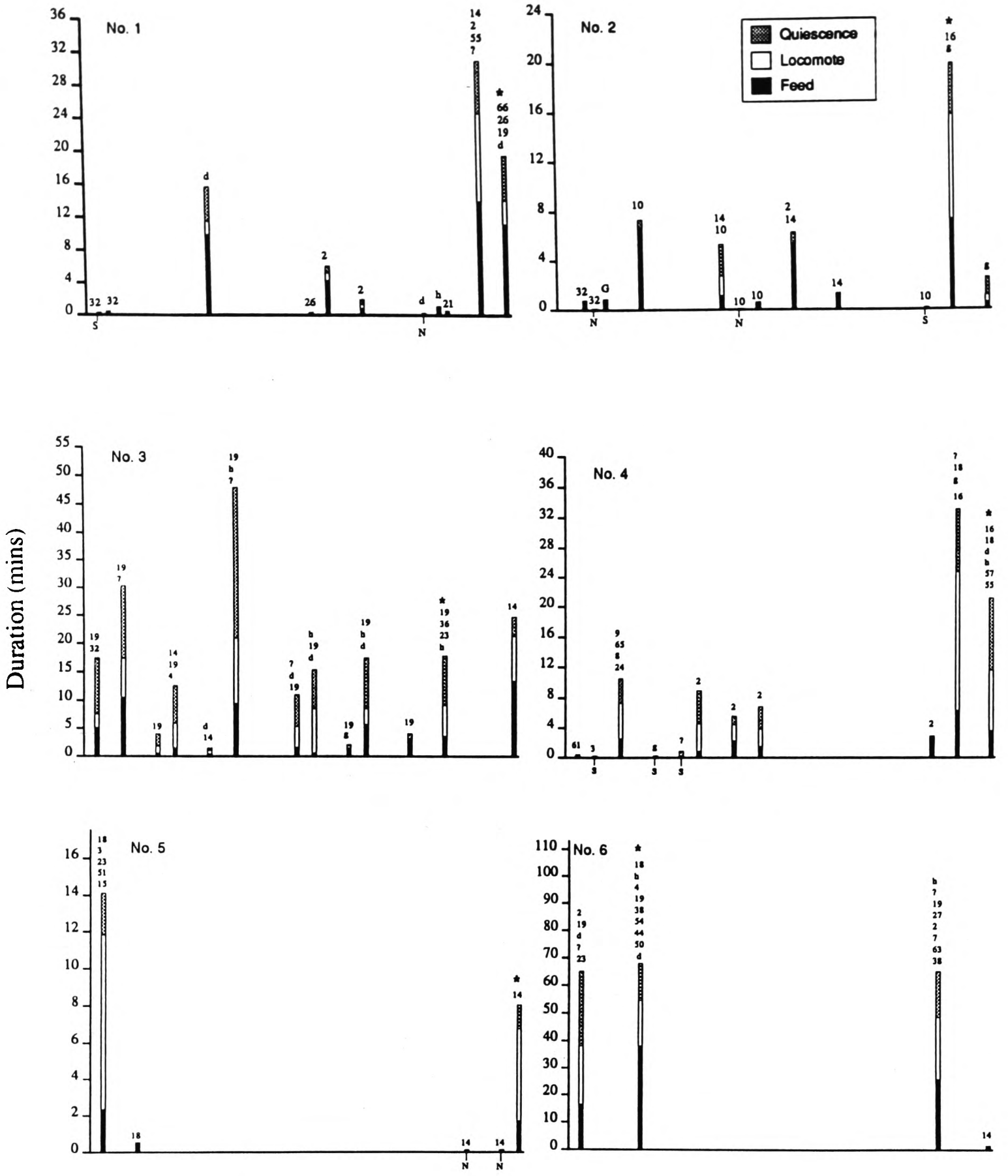
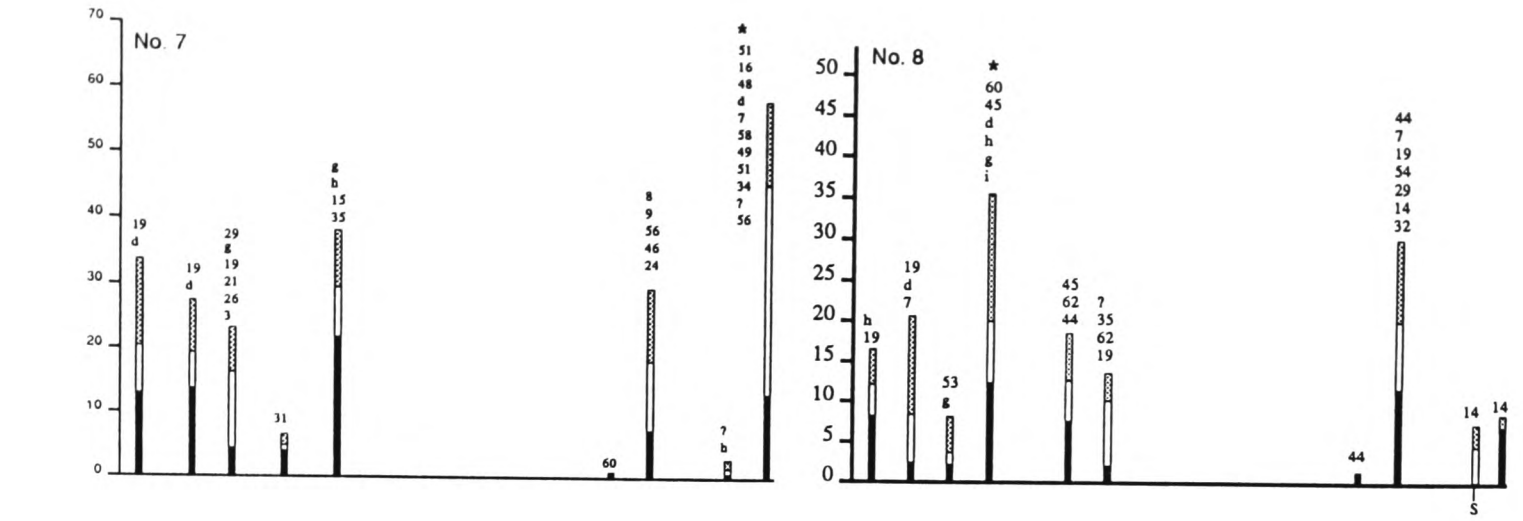
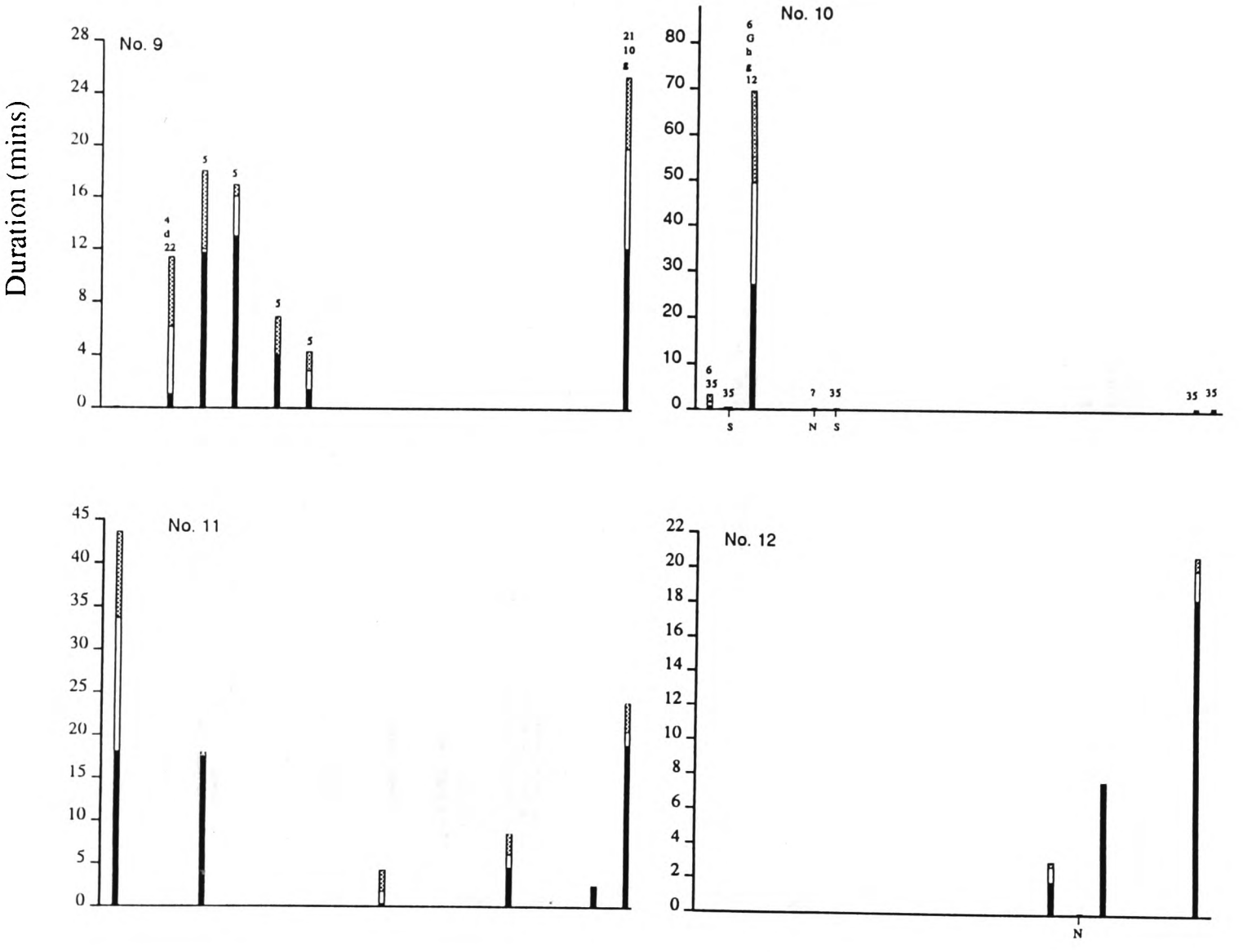


Figure 4. Pattern of meals of 12 *T. eques* observed continuously throughout the day (see overleaf for insects 7-12). Insects 1 to 3 were observed on overcast days and the rest on sunny days. The height of bars indicates meal duration, and the stacks within bars time spent feeding, locomoting and quiescent within meals. An asterisk above the vertical bars identifies individual meals which are detailed in Figure 5. Other symbols above vertical bars refer to the items eaten in each meal, and correspond with the codes in Table 5. Records matching foodplants with feeding events were lost for insects 11 and 12. The symbols S and N along the horizontal axis signify brief periods of feeding termed "snacks" (between 0.15 and 0.29 min.) and "nibbles" (<0.15 min. - see text for further explanation). In each case, the horizontal axis represents approximately 600 minutes.


No. 7
No. 8


Duration (mins)
No. 9
No. 10
No. 11
No. 12

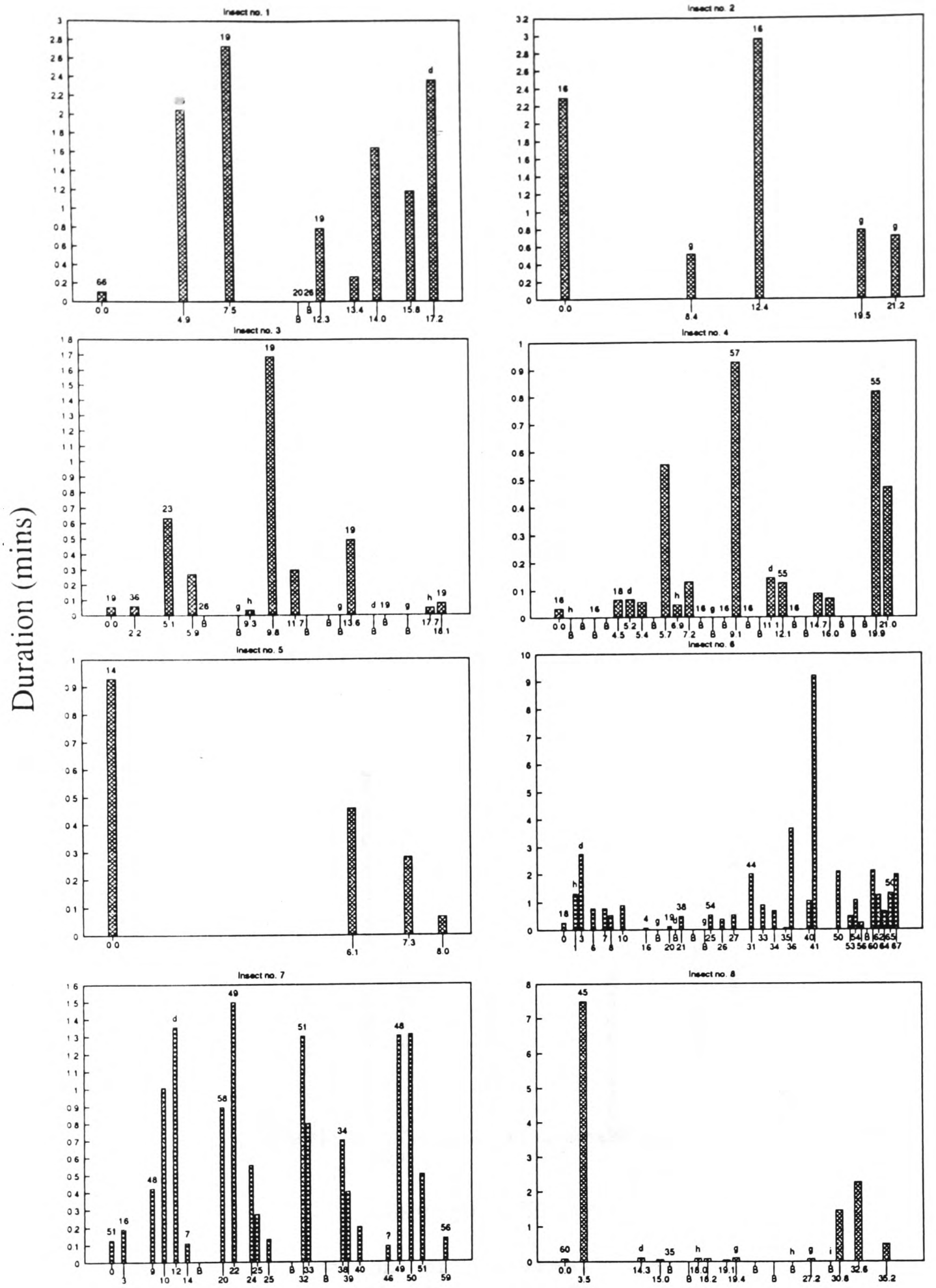


Figure 5. Examples of patterns of feeds within meals. "B" on the horizontal axis signifies rejection at the level of biting. The letters above bars identify the food items and correspond with the codes in Table 5. When a continuous sequence of feeds and/or bites is on the same food item, only the first of these is coded.

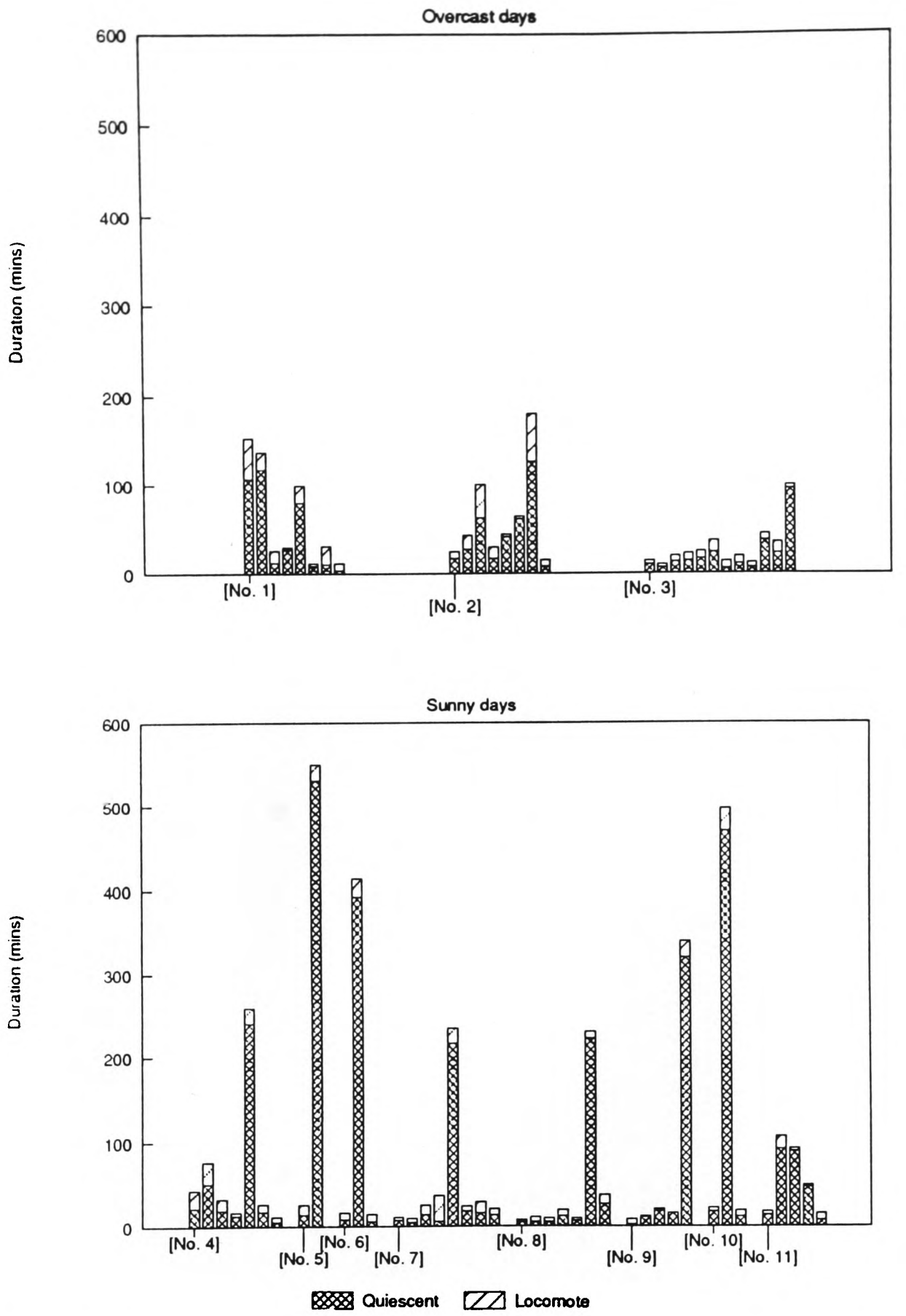


Figure 6. Duration of intermeal intervals of insects observed on overcast and sunny days. Bar height signifies total duration, and the stacks represent time quiescent and locomoting within the intermeal intervals. Bars are placed in order of occurrence.