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# DIET QUALITY INFLUENCES THE $\delta^{13}C$ AND $\delta^{15}N$ OF LOCUSTS AND THEIR BIOCHEMICAL COMPONENTS

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## Summary

**To determine whether relative enrichments of $^{15}N$ and $^{13}C$ in locusts are influenced by diet, locust nymphs were raised from hatchlings to adults on either seedling wheat or maize. Maize provided less hexose sugars and protein per gram than did wheat. Maize also depends on the $C_4$ form of photosynthesis, while wheat uses the $C_3$ form; this difference in photosynthetic pathways produces two distinguishable ranges of $\delta^{13}C$ values.**

**The lower-quality maize diet corresponded to a 5.1‰ increase in animal $\delta^{15}N$, relative to diet, whereas the wheat diet corresponded to an increase of only 2.3‰. The maize-fed animals were more $\delta^{13}C$-depleted in lipid, trehalose and chitin than those fed wheat. The results for $\delta^{15}N$ and $\delta^{13}C$ suggest that substrate recycling occurred on the low-quality maize diet. Consequently, we examined the variations in the isotopic differences between locusts and their diet at the biochemical level.**

Key words: diet, locust, *Locusta migratoria*, substrate recycling, carbon metabolism, nitrogen metabolism.

## Introduction

Many researchers have used the natural abundance levels of the stable isotopes of nitrogen and carbon ($\delta^{15}N$ and $\delta^{13}C$) to explore ecosystem-level energy flows and to describe trophic levels (e.g. Ambrose and DeNiro, 1986; Ambrose, 1990, 1991). The values of $\delta^{15}N$ and $\delta^{13}C$ can also be used to describe the metabolic fates of ingested nitrogen and carbon (Macko *et al.* 1987; Schwarcz and Schoeninger, 1991; Scrimgeour *et al.* 1995). Isotopically distinct diets, having nutritionally different compositions, can be used to study the effects of diet on animal isotope abundance among trophic levels and within the major tissues and chemical constituents of individual animals.

In this context, interpretation of $\delta^{15}N$ and $\delta^{13}C$ values relies on the premise that they are primarily determined by the diet of consumers. The $\delta^{13}C$ of a whole animal is usually very similar to that of the diet (DeNiro and Epstein, 1978). However, body components may show $^{13}C$-enrichment or depletion relative to dietary carbon, e.g. lipid is generally $^{13}C$-depleted relative to the whole-body value, and protein is usually $^{13}C$-enriched. The $\delta^{15}N$ of whole animals is generally $^{15}N$-enriched relative to diet (DeNiro and Epstein, 1981). Hence, on a whole-animal basis, $\delta^{13}C$ is useful for identifying food sources and $\delta^{15}N$ is useful as an indicator of trophic level.

Isotopically discriminating reactions (branch points) are produced at enzymatic steps in metabolic pathways. In physical/chemical systems, the lighter isotope moves more rapidly into the product. In biological systems, the preferential movement of the heavy-to-light isotopes is determined by the enzymatic step. Discrimination is thought to be greatest when the enzyme cleaves a relatively small molecular fragment, as in decarboxylation or deaminations (Webb, 1997). Determination of an isotopic branch point depends on both the amount of discrimination against the heavier isotope and the amount of the element moving through the pathway (Scrimgeour *et al.* 1995; Webb, 1997).

The amino acid pool receives amino acids from dietary protein, from the breakdown of body protein and from the amination of keto acids (Scrimgeour *et al.* 1995). Within this pool, amino acids may be used to synthesize body protein directly or they may be transaminated. Amino acids may also leave the protein pool of the body by excretion (through the synthesis of uric acid) or by deamination to keto acids (Scrimgeour *et al.* 1995). Carbon from carbohydrate and lipid tends to be used or stored directly with a lower proportion of fractionation (Webb, 1997). The direction and magnitude of these fractionating reactions depends on the specific tissue examined and on the nutritional adequacy of the food digested.

Terrestrial vascular plants differ in their $^{13}C/^{12}C$ ratios because of their preferential photosynthetic pathways $C_3$, CAM or $C_4$. Plants using the $C_3$ pathway of photosynthesis (so-named because the first product of photosynthesis is a $C_3$ compound) have $\delta^{13}C$ values varying between −18‰ and −36‰, relative to the universally accepted isotopic standard for $^{13}C/^{12}C$, and the whole-plant $\delta^{13}C$ is heavily dependent on

environmental conditions, such as water availability, light and temperature (O'Leary, 1981). Plants fixing carbon *via* the $C_4$ pathway have a $\delta^{13}C$ range of −7 ‰ to −18 ‰ (Schwarcz *et al.* 1985; Tieszen and Boutton, 1988), and their $\delta^{13}C$ value does not directly reflect the environment, but is a species-specific set point. CAM plants (crassulacean acid metabolism) have $\delta^{13}C$ values intermediate between those of $C_3$ and $C_4$ plants and tend to occur in drought environments. There is a well-tested mechanistic model explaining the $\delta^{13}C$ of plants (Farquhar and Richards, 1984).

There is no such detailed understanding of the causes of variations in plant $\delta^{15}N$. However, among the factors known to influence whole-plant $\delta^{15}N$ are isotopic source variations in soil nitrogen, the influence of microbial associations, such as mycorrhizas (Handley *et al.* 1993; Azcón and Elatrash, 1997; Högberg *et al.* 1994), environmental stresses, such as soil salinity and drought (Handley *et al.* 1996), and symbiotic fixation of atmospheric $N_2$. Nitrogen fixation is a subset of isotopic source changes because, globally, atmospheric $N_2$ is $^{15}N$-depleted relative to whole-soil nitrogen (Raven *et al.* 1993). Many of the same factors influence the intra-plant distributions of $\delta^{15}N$ values so that various parts of the plant may have substantially different $\delta^{15}N$ values (Handley and Raven, 1992). Factors affecting terrestrial plant $\delta^{15}N$ have been reviewed by Handley and Raven (1992), Handley and Scrimgeour (1997) (for whole plants and ecosystems) and Yoneyama (1995) (for the molecular level $\delta^{15}N$).

Locusts are ideal model organisms for exploring stable isotope models of nutrition because their physiology and metabolism have been studied extensively (Simpson and Simpson, 1990; Simpson *et al.* 1995), and several generations of locusts can be reared and followed through their life stages (stadia) in a relatively short time under highly controlled experimental conditions.

In the present study, locusts (*Locusta migratoria*) were raised from the first stadium to adulthood on either a wheat or a maize diet; $\delta^{15}N$ and $\delta^{13}C$ were measured for various body components and compared with the measured values of their plant foods. Wheat was already known to provide a high-quality near-optimum diet and maize a sub-optimal one (Webb, 1997). The aim of this research was to determine whether dietary changes affected metabolic processes, which could be studied by isotopic techniques at the natural abundance level, and how the $\delta^{15}N$ and $\delta^{13}C$ values among biochemical components were affected by diet.

## Materials and methods

### *Elemental analysis and isotope ratio mass spectrometry*

All samples, resulting from the preparations described below, were subjected to continuous-flow isotope ratio mass spectrometry (CF-IRMS) according to the method of Barrie *et al.* (1995) using a Europa model 20-20 CF-IRMS (Europa Scientific Ltd, Crewe, UK) which yields results for %C, %N, $\delta^{13}C$ and $\delta^{15}N$. Sample precisions were ±0.3 ‰ for $\delta^{13}C$ and ±0.4 ‰ for $\delta^{15}N$. The universally accepted standard for $\delta^{13}C$ is a limestone, Pee Dee Belemnite, and that for $\delta^{15}N$ is atmospheric $N_2$ using the relationship:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3 , \quad (1)$$

where $X$ is the heavy isotope of either carbon or nitrogen, $R$ is the ratio of heavy-to-light isotopes and $\delta X$ is measured in parts per thousand (‰).

### *Experimental procedure*

Locusts were reared at the Department of Zoology, Oxford University. All parental stock were reared on a wheat diet. Immediately after hatching, the offspring were fed one of two diets: seedling wheat or seedling maize, for which the isotopic compositions were analyzed. Seedling trays were sampled each week over 1 year. For seedling maize, $\delta^{13}C$ was −13.3±0.5 ‰ and $\delta^{15}N$ was 2.7±0.4 ‰; for seedling wheat, $\delta^{13}C$ was −27.5±0.4 ‰ and $\delta^{15}N$ was 1.5±0.5 ‰.

The insects were kept in crowded conditions at 30 °C under a 12 h:12 h photoregime throughout the experiment. Water and food were always present in excess.

### *Sample preparations*

A subset of 7–15 insects per stadium was taken for isotopic analysis at 3 days post-ecdysis (ecdysis is defined as the time of shedding of the exoskeleton), and whole insects were frozen individually in plastic bags at −50 °C for later determination of quantities and isotopic signatures of lipid, carbohydrate, muscle protein and chitin. Analyses were performed in sequence (see below) on the same individuals. Dry masses were recorded and total lipid content determined. This enabled a comparison of total growth between diets. Samples of frass (faecal output) produced by 1-day-old adults were collected, freeze-dried and processed immediately, as described below.

The experimental treatments were: (1) diet, (2) sex and (3) developmental time (stadium). Developmental time, which compares corresponding life stages, was chosen rather than calendar time, because it was known that the maize-fed locusts would not develop at the same rate as the wheat-fed ones. The experiment lasted 45 days; maize-fed locusts required an average of 38 days to reach adulthood, whereas wheat-fed locusts required only 28 days to proceed through the same number of larval stadia.

Incorporation of new dietary elements is a function of the turnover time of the sampled body component. The faster the turnover, the faster initial carbon and nitrogen are replaced. Here, we define turnover as the time required for an isotopic signal to reach a plateau. Unavoidably, this definition also includes changes due to growth. Because there was no change of diet for the wheat-fed locusts, turnover could be calculated directly only for the maize-fed ones; turnover for the wheat-fed insects was estimated using the results for the maize-fed insects.

To measure lipid content and isotopic values (Loveridge, 1973), each insect was freeze-dried to a constant mass and placed into a sealed glass vial filled with analytical grade

chloroform. The chloroform was replaced at 24 h intervals for 72 h. The resulting chloroform–lipid solution was dried to a constant mass, and the resulting residue consisted solely of lipids, as confirmed by Fourier transform infrared spectroscopy (FT-IR).

A subsample of lipid was transferred in chloroform solution to a tin capsule containing a few milligrams of carbon- and nitrogen-free absorbant ('chromosorb') for ease of handling, from which the chloroform solvent was then evaporated.

Carbohydrate (as trehalose) was extracted (Payne, 1949) from locusts from which whole lipids had previously been extracted. These were placed into 90 % ethanol and refluxed for 3 h. The solution was then filtered and the filtrate evaporated to a constant mass.

Chitin was extracted (DeNiro and Epstein, 1978) by adding 10 % (w/w) NaOH to each insect and heating for 48 h at 105 °C. The NaOH was replaced at 12 h, 24 h and 36 h, and the solutions discarded. The insoluble residues were washed in a series of dilutions of ethanol:water (9:1, 3:1, 1:1 and 1:3, v/v), then washed in deionised water followed by $5\times10^{-2}$ mol $l^{-1}$ HCl, followed by a final wash in deionised water. The residue was then freeze-dried.

Chitin is difficult to extract in pure form (i.e. as polymeric acetylated glucosamine) because, *in vivo*, it is frequently cross-linked to protein and because the extraction procedure is liable to cause deacetylation. Contamination by additional proteins could alter $\delta^{13}C$ and $\delta^{15}N$, whilst removal of the acetyl group could change the $\delta^{13}C$ value. It is, therefore, necessary to show that the data obtained are measured on pure chitin. The products of the extractions were confirmed as pure chitin in three independent ways: (1) examination by Fourier transform infrared spectroscopy (FT-IR), (2) by the C:N ratio and (3) examination for consistency of stable isotope ratio results. Pure chitin has a theoretical C:N ratio of 6.86 (Shimmelmann and DeNiro, 1986), and extracts with a C:N ratio between 6.78 and 7.10 were accepted as pure chitin. The infrared spectra of these extracts corresponded to that for pure chitin, and extracts with deviant C:N ratios (approximately 12 % of the samples) showed contaminated spectra. The C:N ratio of protein is approximately 3–4, and the crude chitin containing the deacetylated form, chitosan, has a C:N ratio of 5.8 (Shimmelmann and DeNiro, 1986).

Muscle and soluble protein (extracted after DeNiro and Epstein, 1978) were compared for isotopic differences. There was no significant difference in the $\delta^{15}N$ or $\delta^{13}C$ values of muscle and soluble protein; therefore, muscle was used to represent the protein component of locusts. Muscle was dissected from the femur, placed in chloroform for 6 h to remove any lipid, washed in deionized water, freeze-dried, then ground to a fine powder. To obtain maximally homogeneous samples, samples having a C:N ratio between 3.7 and 4.2 were selected.

### *Statistical analyses*

The isotopic values for $\delta^{13}C$ and $\delta^{15}N$ were analysed statistically by SPSS, using a two-way analysis of variance (ANOVA). Values are presented as means ± S.E.M. ($N$=10 unless stated otherwise).

## Results

### *Whole locusts*

For wheat-fed *L. migratoria*, there was no significant difference among stadia (Fig. 1A,B; Table 1). Wheat-fed locusts

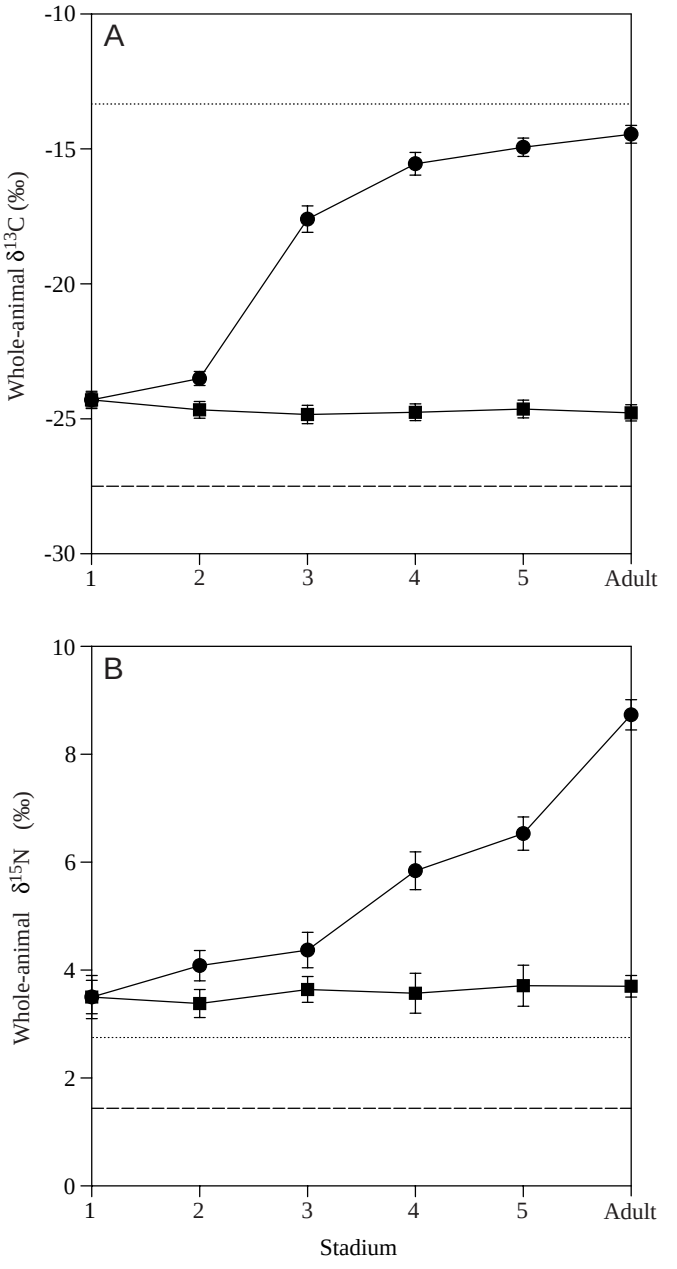


Fig. 1. (A) Graph showing the relationship between diet and whole-animal $\delta^{13}C$ in locusts for each stadium. (B) Graph showing the relationship between diet and whole-animal $\delta^{15}N$ in locusts for each stadium. The dotted line represents the diet value for maize; the dashed line represents the diet value for wheat. Each point represents the mean value for 10 insects. Bars represent ± S.E.M. (squares represent wheat diets, and circles represent maize diets).

Table 1. *Summary of* F *ratios from an ANOVA using age as the main effect for wheat-fed* Locusta migratoria

| | | *F* values | |
|---|---|---|---|
| Source of variation | d.f. | $\delta^{13}$C insect | $\delta^{15}$N insect |
| Main effect | | | |
| Age | 5 | 0.330 | 0.016 |
| Residual | 14 | | |
| Total | 19 | | |

Critical *F* value for significance, $F \geqslant 2.96$.

had mean $\delta^{15}$N and $\delta^{13}$C values of 3.7±0.3‰ and −24.8±0.3‰, respectively. There was no significant difference in isotopic values for males and females. Relative to diet, wheat-fed locust $\delta^{13}$C averaged 2.8‰ higher and $\delta^{15}$N averaged 2.3‰ higher.

For maize-fed locusts, however, both $\delta^{15}$N and $\delta^{13}$C varied significantly across stadia (Fig. 1A,B). Whole adults of both sexes fed maize from the first stadium onwards had mean $\delta^{15}$N and $\delta^{13}$C values of +7.7‰ and −15.8‰. Hence, whole locusts were 2.5‰ more $^{13}$C-depleted than their bulk diet and 5.1‰ more $^{15}$N-enriched than their bulk diet. $\delta^{13}$C values plateaued by the fourth stadium; $\delta^{15}$N values did not plateau during the course of the experiment.

Locust size gives an indication of the adequacy of diet. Whole adult locusts of both sexes (Fig. 2) were heavier and had a larger lipid content when fed wheat than when fed maize. For both diets, females were heavier than males, but contained proportionately the same amount of lipids. The only significant treatment effect was diet (Table 2).

### *Biochemical components of locusts*

#### *Muscle*

Both dietary treatments resulted in isotopic enrichment ($^{13}$C and $^{15}$N) of muscle relative to bulk diet (Fig. 3A,B; Table 3).

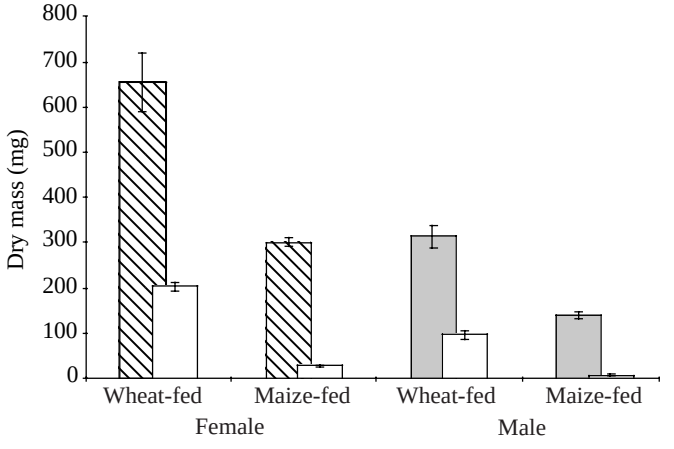


Fig. 2. Dry masses (mean ± S.E.M., *N*=10 insects) of male grey columns) and female (hatched columns) locusts fed on the wheat or maize diet. Lipid content (open columns) is shown in front of corresponding dry mass. All locusts were 7-day-old adults.

Table 2. *Summary of* F *ratios from a two-factor ANOVA using stadium and diet as the main effects for wheat- and maize-fed* Locusta migratoria

| | | *F* values | |
|---|---|---|---|
| Source of variation | d.f. | $\delta^{13}$C insect | $\delta^{15}$N insect |
| Main effects | | | |
| Diet, D | 1 | 8400.42*** | 635.56*** |
| Stadium, S | 5 | 739.84*** | 113.58*** |
| Interaction | | | |
| D×S | 5 | 787.48*** | 92.38*** |
| Residual | 19 | | |
| Total | 30 | | |

***$P$<0.001.

The adult wheat-fed locusts were, on average, $^{13}$C-enriched relative to their diet by 3.7±0.3‰ and $^{15}$N-enriched relative to their diet by 3.1±0.3‰. The adult maize-fed locusts were, on average, $^{13}$C-enriched relative to their diet by 0.7±0.2‰ and $^{15}$N-enriched relative to their diet by 7.8±0.2‰.

There was no developmental change in muscle $\delta^{13}$C or $\delta^{15}$N for locusts fed the wheat diet (Fig. 3A,B). Locusts on the maize diet, however, experienced increasing enrichment of $^{13}$C through the first three stadia, and a continuously increasing enrichment of $^{15}$N through the entire life cycle.

#### *Chitin*

Relative to diet, wheat-fed adults were $^{13}$C-enriched and maize-fed adults were $^{13}$C-depleted. For wheat-fed adults, the mean $^{13}$C-enrichment relative to diet was 2.2±0.2‰, and for maize-fed adults this value was −0.2±0.1‰. In adults, the mean chitin $\delta^{15}$N was $^{15}$N-depleted relative to diet for both wheat- and maize-fed locusts by 3.7±0.3‰ and 4.2±0.2‰, respectively.

The turnover for chitin carbon was rapid. Shortly after the first moult and following the initial dietary switch to maize, locusts achieved a fully maize-derived $^{13}$C isotopic signature (Fig. 4A). Chitin $\delta^{15}$N values showed little change with age; hence, nitrogen turnover time could not be estimated graphically (Fig. 4B).

#### *Lipid*

In adult wheat-fed locusts, lipid (Fig. 5) was $^{13}$C-depleted (by 1.3±0.2‰) relative to diet; in maize-fed locusts, the equivalent $^{13}$C-depletion for lipid was 9.4±0.2‰. Turnover of lipid carbon was slow (see Table 4).

#### *Trehalose*

Relative to dietary carbon (Fig. 6), the mean $\delta^{13}$C value for trehalose of wheat-fed adults was only slightly $^{13}$C-enriched (0.3±0.2‰). This enrichment was, however, statistically significant at $P$=0.001. However, in maize-fed locusts, the mean $\delta^{13}$C of trehalose was greatly $^{13}$C-depleted relative to diet (5.5±0.3‰).

Trehalose $\delta^{13}$C showed little variation across stadia when

locusts were fed wheat (Fig. 6; Table 3) because there was no change in diet. For maize-fed locusts, the turnover time of trehalose was four stadia.

*Faeces*

Frass (faecal) samples were collected from adult individuals, three samples from nine adults of the maize treatment and three samples from three adults of the wheat treatment.

Values for $\delta^{15}N$ and $\delta^{13}C$ of frass from the maize treatment were +6.3±2.3 ‰ and −14.3±1.9 ‰. Within this treatment, frass was on average 3.6 ‰ $^{15}N$-enriched relative to diet and 1.0 ‰ $^{13}C$-depleted. For the wheat treatment, frass $\delta^{15}N$ and $\delta^{13}C$ values were +2.6±0.2 ‰ and −26.9±1.0 ‰, respectively. These corresponded to a mean $^{15}N$-enrichment of 1.1 ‰ and a mean $^{13}C$-enrichment of 0.6 ‰ relative to diet.

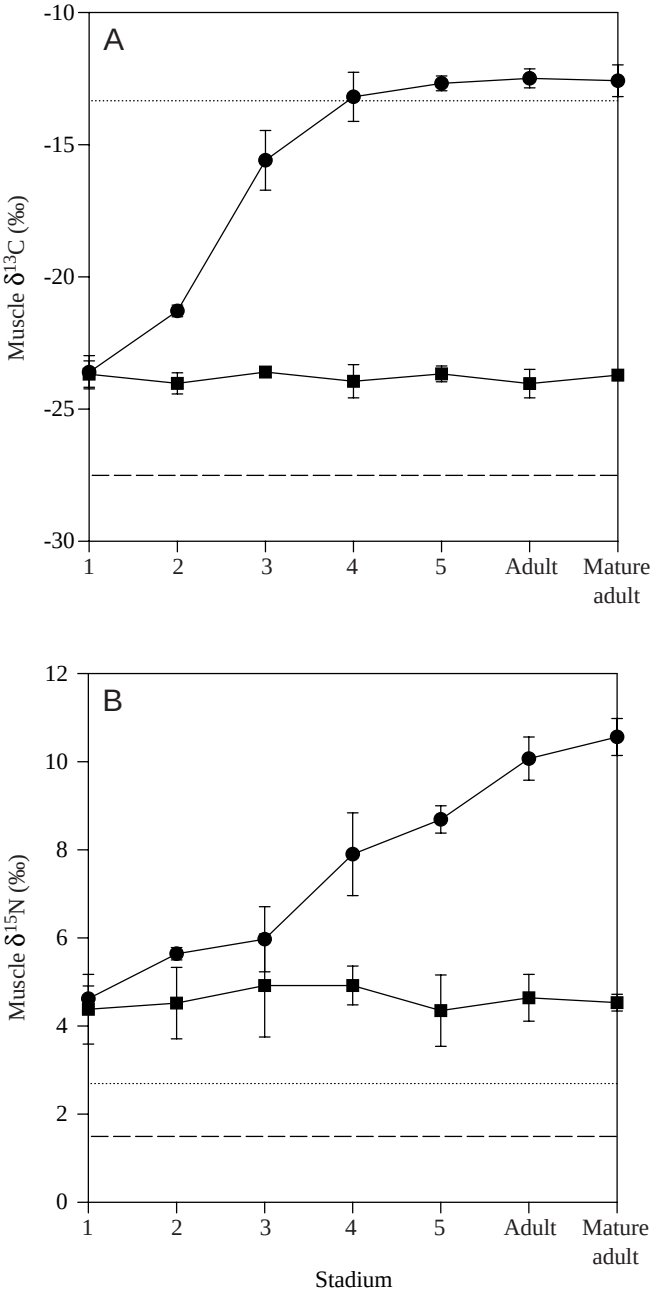


Fig. 3. (A) Graph showing the relationship between diet and muscle $\delta^{13}C$ for each stadium. (B) Graph showing the relationship between diet and muscle $\delta^{15}N$ for each stadium. The dotted line represents the diet value for maize; the dashed line represents the diet value for wheat. Each point represents the mean value for 10 insects. Bars represent ± S.E.M. (squares represent wheat diets, and circles represent maize diets).

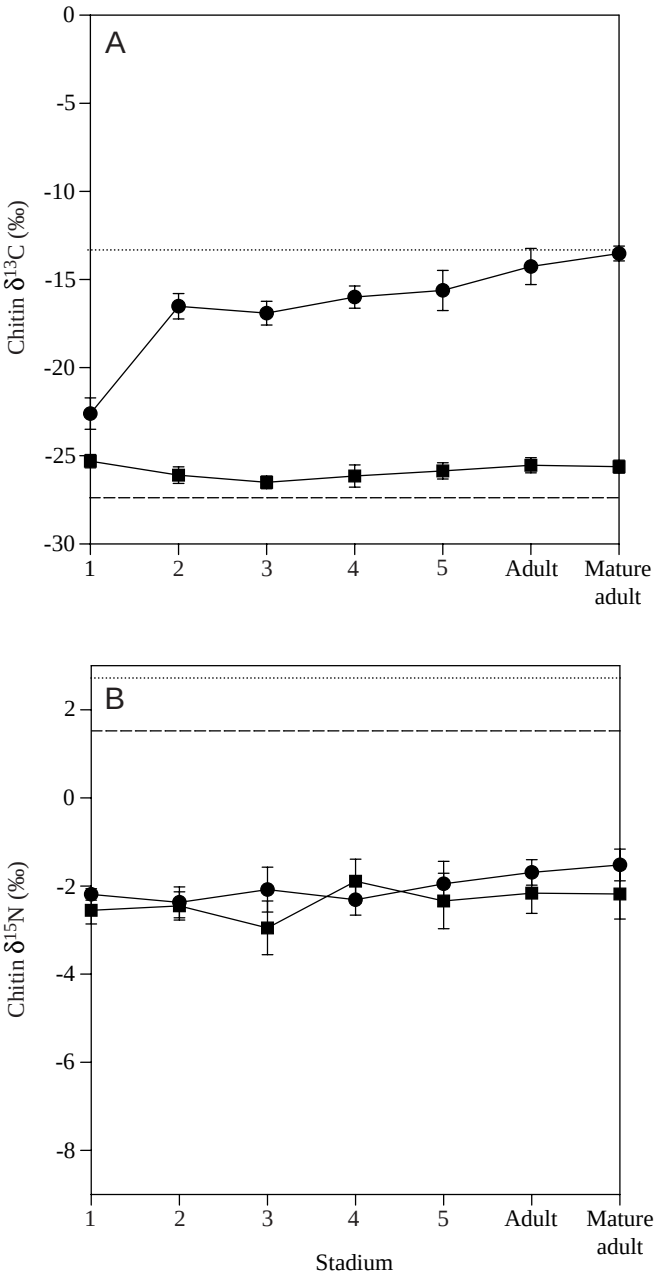


Fig. 4. (A) Graph showing the relationship between diet and chitin $\delta^{13}C$ for each stadium. (B) Graph showing the relationship between diet and chitin $\delta^{15}N$ for each stadium. The dotted line represents the diet value for maize; the dashed line represents the diet value for wheat. Each point represents the mean value for 10 insects. Bars represent ± S.E.M. (squares represent wheat diets, circles represent maize diets).

Table 3. *Summary of* F *ratios from a two-factor ANOVA using stadium and diet as the main effects for wheat- and maize-fed* Locusta migratoria

| Source of variation | d.f. | *F* values | | | | | |
|---|---|---|---|---|---|---|---|
| | | $\delta^{13}$C lipid | $\delta^{13}$C trehalose | $\delta^{13}$C muscle | $\delta^{15}$N muscle | $\delta^{13}$C chitin | $\delta^{15}$N chitin |
| Main effects | | | | | | | |
| Diet, D | 1 | 1585.64*** | 1063.78*** | 6103.86*** | 726.47*** | 6096.89*** | 18.88*** |
| Stadium, S | 6 | 100.45*** | 6.76*** | 261.71*** | 57.58*** | 76.74*** | 5.95*** |
| Interaction | | | | | | | |
| D×S | 6 | 238.79*** | 187.19*** | 261.63*** | 60.51*** | 73.43*** | 4.32 ** |
| Residual | 128‡ | | | | | | |
| Total | 141‡ | | | | | | |

Results are shown for lipid, trehalose, muscle and chitin.
***$P$<0.001; **$P$<0.01.
‡ Residual and total for lipid and trehalose are 107 and 119, respectively

*Whole-animal isotopic mass balance*

The following isotopic mass balance predicts, on the basis of protein and chitin analyses, that adult wheat-fed insect $\delta^{15}$N should be:

$$\delta X_{\text{whole insect}} = (\delta X_{\text{constituent a}} \times f_{\text{a}}) + (\delta X_{\text{constituent b}} \times f_{\text{b}}) + \ldots . \quad (2)$$

where $\delta X$ is the natural abundance of either $^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N and $f$ is the fraction of total insect carbon or nitrogen in a given constituent. The actual measured whole-insect value of 3.4‰ was close to the predicted value of 3.7±0.3‰. The corresponding predicted and measured values for whole insects fed maize were 8.4‰ and 8.7±0.1‰, respectively. Hence, we may assume that protein and chitin nitrogen together account for the vast majority of body nitrogen.

Similarly for carbon, an isotopic mass balance predicted that whole insects (wheat-fed) should have a $\delta^{13}$C value of –26‰; this closely resembled the measured value of –24.8±0.3‰, confirming that trehalose, chitin, protein and lipid accounted for the significant carbon pools. The corresponding predicted and measured values for whole insects (maize-fed) were –13.9‰ and –14.4±0.3‰, respectively.

*Carbon turnover*

The diet of wheat-fed insects remained constant; therefore, turnover times of tissues could be calculated directly for maize-fed insects only since the wheat-fed insects showed no variation across the stadia. $\delta^{15}$N values for wheat-fed insects did not plateau but showed a slight increase through the life of the insect.

As shown in Table 4, chitin carbon had the fastest turnover rate during development, and whole-animal, protein and trehalose carbon showed approximately the same rates of turnover.

The turnover for chitin carbon was rapid. From Fig. 4A, carbon turnover was estimated to be approximately 8 days for chitin of locusts fed on maize. Turnover of muscle carbon was

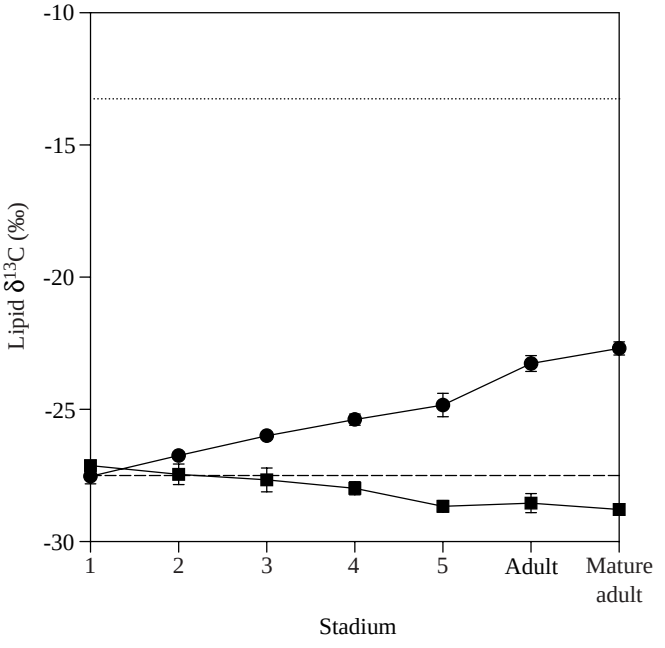


Fig. 5. Graph showing the relationship between diet and lipid $\delta^{13}$C for each stadium. The dotted line represents the diet value for maize; the dashed line represents the diet value for wheat. Each point represents the mean value for 10 insects. Bars represent ± S.E.M. (squares represent wheat diets, circles represent maize diets).

Table 4. *Turnover times (developmental and days) for whole insects and biochemical constituents*

| Sample | Stadium | Turnover time (days) | |
|---|---|---|---|
| | | Maize-fed | Wheat-fed |
| Whole insect | 4 | 24 | 18 |
| Protein | 3–4 | 22 | 16 |
| Chitin | 2 | 8 | 5 |
| Lipid | Adult | 38 | 27 |
| Trehalose | 4 | 24 | 18 |

Results for maize-fed insects were used to calculate the turnover time (in days) for wheat-fed insects.

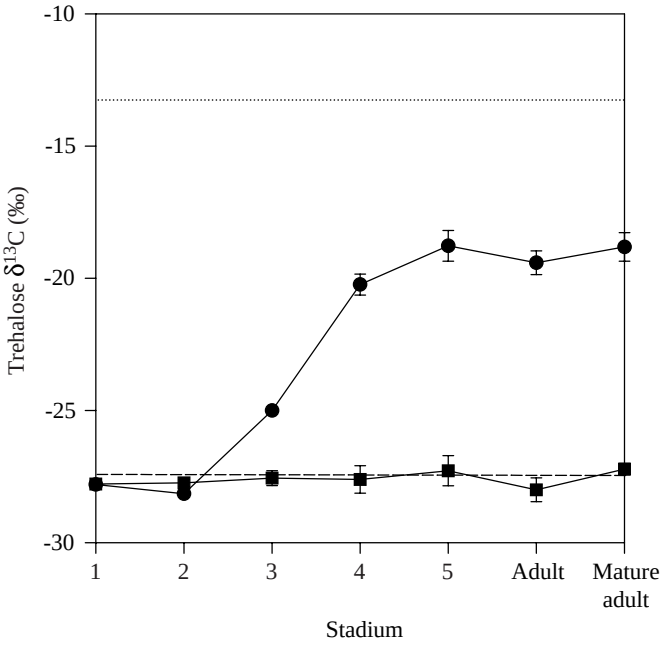


Fig. 6. Graph showing the relationship between diet and trehalose $\delta^{13}C$ for each stadium. Each point represents the mean value for 10 insects. Bars represent ± S.E.M. (squares represent wheat diets, circles represent maize diets).

approximately 3–4 stadia (Fig. 3A). For maize-fed locusts, the turnover time of trehalose was four stadia (Fig. 6), similar to that of muscle. The graphically estimated turnover time for locust lipid from maize-fed insects was approximately 38 days (Fig. 5), the time required for reaching adulthood.

The use of developmental stages (stadia) synchronises the turnover rates between diets. Thus, the turnover times for wheat-fed insects and their biochemical constituents could be calculated from the turnover times of maize-fed insects since turnover times are related to stadia duration (Webb, 1997); these values are shown in Table 4.

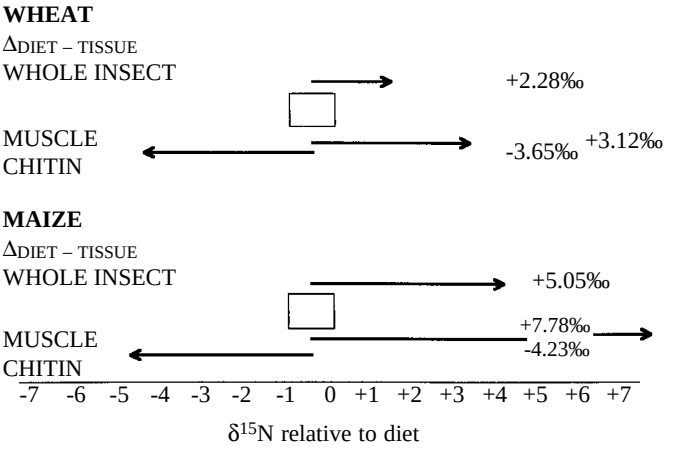


Fig. 7. $\delta^{15}N$ and the relationship to diet (diet has been normalized).

The relationship between $\delta^{15}N$ and diet is summarized in Fig. 7 and that between $\delta^{13}C$ and diet is summarized in Fig. 8.

## Discussion

### *$\delta^{13}C$* versus *diet and development stage*

The parents of the experimental locusts were reared on wheat, leaving a residual isotopic and compositional wheat signal in the offspring. For $\delta^{13}C$ of whole locusts, the initial wheat label was lost after the first four stadia of growth in the maize-fed animals. Because there was no change of diet for the wheat-fed animals, it was not possible to detect the disappearance of the initial isotopic signal. Turnover rates were estimated graphically by noting the point at which the $\delta^{13}C$ *versus* stadium curve for the maize-fed insects reached a plateau. Chitin showed the fastest turnover of any constituent examined. Whole insects, trehalose and protein all turned over at approximately the same rates. Chitin, therefore, is a good indicator of very recent dietary source carbon for growing

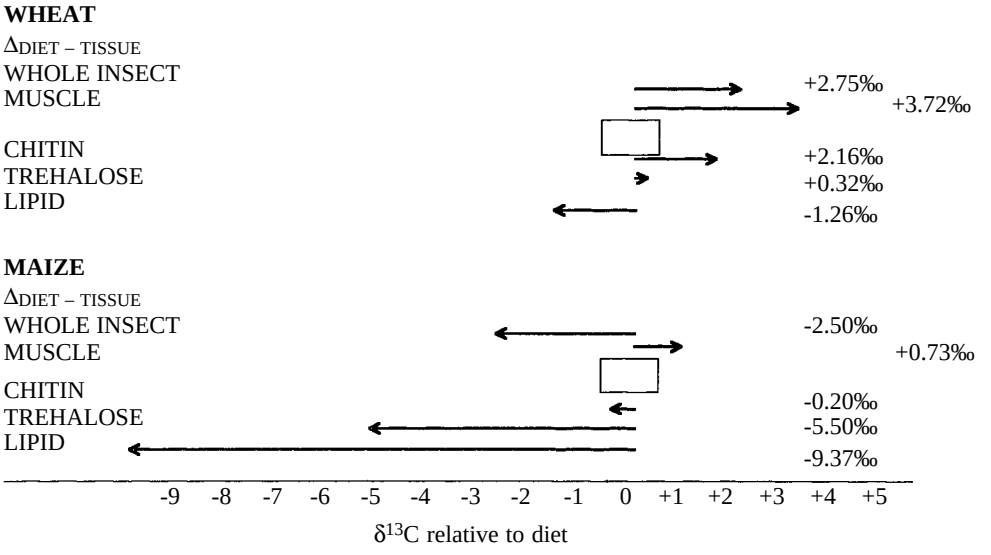


Fig. 8. $\delta^{13}C$ and the relationship to diet (diet has been normalized).

insects and whole insects, whereas trehalose and protein are better indicators for integrated, long-term dietary carbon sources. Lipids turned over very slowly under the maize diet; however, it is known that with a carbohydrate-deprived diet, such as this one, locusts do not store large quantities of lipids (Zanotto *et al.* 1993). Therefore, these data may not be representative of lipid turnover under natural conditions.

### *δ15N* versus *diet and developmental stage*

Whereas $\delta^{13}C$ is frequently useful as a tracer of dietary carbon source, $\delta^{15}N$ is usually associated with a $^{15}N$-enrichment (average 3–5 ‰) at each change of trophic level, termed 'stepwise enrichment' (Minagawa and Wada, 1984). It is well documented (Hobson *et al.* 1993) that starvation or nitrogen deficiency increases this stepwise enrichment substantially. As explained by Scrimgeour *et al.* (1995), amino acids derived from protein breakdown are deaminated to keto acids, a portion of which are reaminated and reincorporated into body tissue. Each transamination results in nitrogen isotope discrimination, with excreted nitrogen being depleted in $^{15}N$ and the retained amino acids being correspondingly enriched. Fractionation factors for specific transaminations are given by Handley and Raven (1992; their Table 4). Because the rate of incorporation of these recycled amino acids depends on the turnover rate of the relevant tissues, the largest $^{15}N$ enrichments would be expected in tissues with the fastest turnover. In the present study, chitin showed the fastest turnover and was $^{15}N$-depleted relative to whole insects (Fig. 7).

However, chitin is atypical of the process described above, because it constitutes substantial body reworking with large quantities (but not all) of each exoskeleton being reabsorbed and a new exoskeleton being formed initially (at least partially) from resorbed old chitin, with the remainder of the new exoskeleton being formed *de novo* (Retnakaran, 1986; Muzzarelli *et al.* 1986). Hence, each exoskeleton is a mixed pool of old and new nitrogen, formed against the background of changing amounts of incorporated dietary nitrogen. In addition, all the chitin nitrogen in each exoskeleton is derived from excretory ammonia (Retnakaran, 1986; Muzzarelli *et al.* 1986), which is known to be isotopically light compared with dietary nitrogen (Minagawa and Wada, 1984).

Equally atypical were the $^{15}N$ enrichments of frass relative to diet. In most animals studied, faecal material is normally $^{15}N$-depleted relative to diet (Lajtha and Michener, 1994), with the isotopic branch point occurring between the whole animal and the excreta. In the case of locusts, there are at least two isotopic branch points in the excretion pathway: (1) ammonia nitrogen to chitin (Muzzarelli *et al.* 1986) and (2) residual uric acid nitrogen to waste uric acid (Salway, 1994). Because the ammonia nitrogen used to form chitin is $^{15}N$-depleted, the residual excretory nitrogen pool, used to form uric acid, is correspondingly $^{15}N$-enriched, thus explaining the unusual results for frass $\delta^{15}N$.

Maize-fed, whole-insect $\delta^{13}C$ reached a plateau early in growth, indicating a replacement of the initial carbon by new dietary carbon. $\delta^{15}N$, however, continued to increase throughout the experiment. Because maize-fed insects were given a suboptimal diet, internal recycling of body nitrogen, with an attendant increase in $^{15}N$ *via* selective $^{14}N$ excretion, is the simplest explanation for this result. This phenomenon under deprived diet has been documented, for example by Hobson *et al.* (1993), who examined the effect of nutritional stress in juvenile Japanese quail (*Coturnix japonica*) and Arctic-nesting female Ross' geese (*Chen rossii*) during egg laying and incubation. Elevated $\delta^{15}N$ values in both species were observed and attributed to reduced nutrient intake.

A calculated isotopic mass balance explained the whole-insect $\delta^{15}N$ in terms of the isotopic enrichment of protein balanced by the isotopic depletion of chitin; similarly, an isotopic mass balance for whole-insect carbon verified that the major carbon pools had been measured.

In summary, the present study provides measurements of $\delta^{13}C$ and $\delta^{15}N$ values for the main chemical classes in the tissues of *L. migratoria* and relates them to the values in the diet of the insect. There were no significant carbon and nitrogen isotopic differences between males and females on the same diet. The turnover rates for the various tissues were established using only $\delta^{13}C$, since $\delta^{15}N$ did not reach a plateau. Chitin $^{15}N$ showed increased depletion relative to diet, while frass $^{15}N$ became increasingly enriched, suggesting that the acetylated amino group on the chitin molecule was formed from excretory ammonia. The protein $\delta^{15}N$ of maize-fed insects was indicative of body-protein recycling. Body-protein recycling may also be supported by the $\delta^{13}C$ of biochemical components from insects fed the maize diet (extreme depletion in lipid and trehalose $^{13}C$ from maize-fed insects in comparison with wheat-fed insects relative to diet). The results show that not only dietary isotopic composition but also dietary nutritional quality can determine the spacing between stable isotope ratios in the tissues of a consumer. The next step is to use artificial diets, varying in proportion and isotopic signal of protein and carbohydrate, to test whether isotopic ratios and turnover rates are affected by diet quality.

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