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# Target for lipid-to-carbohydrate intake minimizes cost of growth

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Many theoretical treatments of foraging use energy as currency, with carbohydrates and lipids considered interchangeable as energy sources. However, herbivores must often synthesize lipids from carbohydrates since they are in short supply in plants, theoretically increasing the cost of growth. We tested whether a generalist insect herbivore (*Locusta migratoria*) can improve its growth efficiency by consuming lipids, and whether these locusts have a preferred caloric intake ratio of carbohydrate to lipid (C : L). Locusts fed pairs of isocaloric, isoprotein diets differing in C and L consistently selected a 2C : 1L target. Locusts reared on isocaloric, isoprotein 3C : 0L diets attained similar final body masses and lipid contents to locusts fed the 2C : 1L diet, but they ate more and had a ~12% higher metabolic rate, indicating an energetic cost for lipogenesis. These results demonstrate that some animals can selectively regulate carbohydrate-to-lipid intake and that consumption of dietary lipids can improve growth efficiency.

## 1. Introduction

The primary task of optimal foraging theory is to use techniques of mathematical optimization to make predictions about the foraging behaviour of animals, typically assuming they should favour efficiency by maximizing energy intake rate and minimizing foraging costs [1–4]. Optimal foraging theory and models have been widely used in behavioural ecology and have even been applied to human behaviour and social sciences [5–7]. However, there are limitations where nutritional behaviour cannot be completely predicted [4,8,9]. Studies using the geometric framework for nutrition provide additional insight and have revealed that balancing macronutrients, specifically protein relative to non-protein (NP) energy (carbohydrates and lipids) is a strong driver of foraging behaviour across the animal kingdom (reviewed in [10,11]). In some cases, omnivorous and carnivorous vertebrates and some arachnids have been shown to regulate all three macronutrients (proteins, carbohydrates and lipids) to specific caloric ratios (P : C : L) [12–15]. However, whether the capacity to regulate carbohydrate-to-lipid intake occurs broadly across animals, and the potential selective forces driving carbohydrate-to-lipid (C : L) regulation, has been little explored. Here, we use the geometric framework for nutrition to test whether an insect herbivore can regulate C : L and, if so, whether C : L intake regulation can minimize the energy costs of consumption and growth.

Many theoretical treatments of foraging, including optimal foraging theory and geometric framework for nutrition, often consider carbohydrates and lipids as interchangeable energy sources because lipids can be synthesized from carbohydrates. However, studies using mice models together with $C^{14}$ tracing, metabolic measurements and stoichiometry estimate that 24–28% of ingested carbohydrate energy is lost when converted to fat through de novo

synthesis [16]. In contrast, only 2–7% of ingested lipid energy is lost through the process of building lipid storage [16], suggesting that the capacity to find and consume the lipid needed for lipid growth should provide an energetic benefit over de novo lipogenesis. For herbivores, some studies have explored their requirements for essential fatty acid [17–19] and sterol (which cannot be synthesized by some herbivores [20,21]). However, lipid consumption as an energy source has been largely ignored in the geometric framework for nutrition studies of herbivores because it is thought that carbohydrates make up the majority of the NP energy in foliage [10,22–24]. However, many herbivores can have access to lipid-rich food sources, such as seeds [25], suggesting they may differentiate between C and L food sources if it is important for fitness.

Locusts are among the most important agricultural pests globally and they have an impressive capacity for long-distance flight, including reports of crossing the Atlantic Ocean [26]. These herbivores use lipids as a primary fuel during long flights and body lipids make up 15–35% of their dry body mass [27,28]. Whereas it was assumed that locusts and other herbivores build lipid stores predominantly from ingested carbohydrates, many locust species have been observed feeding on seeds [29], which are generally rich in lipids and fatty acids [30,31]. These observations, combined with the high lipid requirements of locust flight [28,32,33] and the potentially high energy cost of de novo lipogenesis [16,34], led us to hypothesize that locusts regulate their energy C to L intake ratio to preferentially synthesize storage lipids from ingested lipids, increasing energetic efficiency.

Combining approaches and predictions from the geometric framework for nutrition and optimal foraging theory, we hypothesized that (i) synthesizing lipids from carbohydrates is sufficiently costly that herbivores who require substantial lipid reserves have evolved the capacity to sense and regulate relative lipid intake; and (ii) access to lipids in the diet will improve the efficiency of lipid storage accumulation. To test whether locusts can regulate C : L intake, we designed choice experiments where individual locusts were offered two complementary iso-caloric, iso-protein diets with different carbohydrate-to-lipid energy ratios. To test whether lipid intake improves lipid accumulation efficiency, we compared development performance, body lipid growth rate and metabolic rates between locusts that were reared on confined isocaloric diets with and without lipids.

## 2. Material and methods

### (a) Animals

Lab experiments were conducted at Arizona State University (ASU), using a lab colony of *Locusta migratoria* (see [28] for more population details). Locusts were reared in crowded cages on wheat grass, romaine lettuce and wheat bran ad libitum. The relative humidity was 30.0 ± 0.5% and air temperatures were 34 ± 0.5°C during the day and 25.0 ± 0.5°C during the night (14L : 10D photoperiod), with supplementary heat supplied by incandescent 60W light bulbs during light phases so that locusts could thermoregulate to higher body temperatures. We used male and female final (fifth) instar nymphs for all our experiments, which were collected, sexed, weighed and assigned randomly to each experiment within 3–5 h following moult. In all experiments, each locust was housed individually in a clear acrylic cage (19 cm × 10.5 cm × 14 cm).

### (b) Choice experiments: do locusts regulate their carbohydrate-to-lipid energy intake ratio?

To study whether locusts regulate their C : L energy intake ratio, we designed dry iso-caloric artificial diets (see the §2d and electronic supplementary material, table S1 for full ingredients list). We supplied a pair of pre-weighed dry artificial diet dishes to each locust. Half the locusts were given a dish of 3P : 5C : 1L (protein : carbohydrate : lipid energy ratio) diet and a dish of 3P : 1C : 5L diet, and another half were provided dishes of 3P : 5C : 1L and 3P : 2C : 4L diets (25 individuals for each diet pair, from each sex). For these diets, ratios refer to the relative proportion of all caloric energy in that diet provided, instead of the mass ratios, which are frequently used in the geometric framework literature (see electronic supplementary material, table S1 for full details on the diets). In addition to artificial diets, we provided ad libitum water in a tube with a cotton plug. After 3 days, we replaced the diet dishes with new pre-weighed dishes. The consumption of each diet was measured as the difference between the initial and final dry mass in each diet dish (following drying for 24 h at 60°C, in an ED56-UL oven, Binder, Tuttlingen, Germany). To calculate the energy consumption of each macronutrient, we multiplied each individual's diet consumption by the energy density ($J \cdot g^{-1}$) of that macronutrient (carbohydrates, lipids or proteins) in the diets. To test whether the specific lipid source affects how locusts regulate C : L consumption, perhaps owing to different smells or tastes, we carried out this experiment three times using three different oil sources (canola, sunflower and grapeseed). We chose these three specific oils because their compositions of fatty acids are relatively similar [35,36]. Note that even the zero lipid diets contain some lipids in the form of essential fatty acids, but the concentrations of these are so low that their effect on energy content is less than 3% of the total energy content.

### (c) Single diet experiments: how do locusts perform on diets without lipids (as a macronutrient) compared with diets with the preferred carbohydrate-to-lipid caloric ratio?

In this experiment, we measured developmental time, survival, energy consumption rates and body lipid accumulation rate when locusts were confined to a single diet during fifth instar nymphal development. We designed a new artificial diet, 3P : 4C : 2L (energy ratio; see electronic supplementary material, table S1 for the full recipe), based on the self-selected C : L intake target energy ratio found in the choice experiment, and compared locust developmental performance on this 'ideal C : L diet' with

that on a diet containing only carbohydrates as their NP energy source (3P : 6C : 0L energy ratio). Pre-weighed diet dishes were inserted into each individual cage (30 individuals in each diet treatment from each sex), replaced after 3 days with new fresh dishes and removed at the end of the experiment when the locusts moulted to adults.

## (d) Respirometry

We measured oxygen consumption and carbon dioxide production of 5–6-day-old fifth instar locust nymphs reared on confined diets (20 individuals in each diet treatment from each sex). We carried out constant volume respirometry using a FoxBox field respirometry system (Sable Systems International, Las Vegas, NV, USA). The span of the oxygen analyser was calibrated several times a day by flushing the system with dry, $CO_2$-free air for at least 20 min. The calibration of the $CO_2$ analyser was carried out at the ASU lab using pure nitrogen gas and two certified calibration tanks (252 ± 1 and 1010 ± 1 ppm of $CO_2$ balanced in $N_2$, factory certified). We used 60 ml syringes closed with three-way valves as metabolic chambers.

After inserting the nymph into the metabolic chamber, the chamber was flushed with dry, $CO_2$-free air for 1 min at a flow rate of 500 ml $min^{-1}$. The syringe was then sealed and placed at the rearing temperature for ~60 min (using environmental chamber; model MIR554, Panasonic, Osaka, Japan), after which 40 ml of air were injected into a stream of dry, $CO_2$-free air, at a flow rate of 500 ml $min^{-1}$, which passed through a magnesium perchlorate column, $CO_2$ analyser (FoxBox), an Ascarite®/silica gel column and then an $O_2$ analyser (FoxBox). We corrected the metabolic chamber volume by subtracting the animal volume from it, which was calculated from animal mass assuming a density of 1. Baselining was repeated between individual measurements by passing dry $CO_2$-free air directly through the analysers. Data collection and analyses were carried out using a UI-3 data acquisition interface and Expedata software (Sable Systems International).

## (e) Total body lipid extraction

We used a chloroform extraction technique to measure the lipid content [37] of locusts that were reared on confined diets after they moulted to adults. We sacrificed ~30 (for each sex) freshly moulted fifth instar nymphs taken directly from the colony to estimate initial body lipid content. We calculated lipid accumulation rate (g $d^{-1}$) from the change in body lipid mass on each diet divided by number of days on the diet treatment.

## (f) Artificial diets

We made dry iso-caloric artificial diets, which varied in protein : carbohydrate : lipid energy (caloric) ratios as follows: 3P : 1C : 5L, 3P : 5C : 1L, 3P : 2C : 4L, 3P : 4C : 2L, and 3P : 6C : 0L (see electronic supplementary material, table S1 for additional details of the diet contents). Note that the energy in protein always accounted for one-third of the total energy in each diet, which is in the range of self-selected targets of other grasshopper and locust species [22,23,38]. Our diets were modified from standard artificial locust diets [39], with proteins provided as a mix of three parts casein, one part peptone and one part albumen, by dry mass. Carbohydrates were provided using an equal mix of sucrose and dextrin, by dry mass. We used plant-based lipid sources (canola oil, sunflower oil and grapeseed oil) for each of the three different choice experiments, and only canola oil for the no-choice experiment. To keep all diets iso-caloric with specific energy-based ratios, we calculated the amount of each ingredient using the following conversions for energy density: 4.1 kcal $g^{-1}$ for carbohydrates and proteins and 8.8 kcal $g^{-1}$ for lipids. All diets contained equal amounts of salt ions and vitamins, as is provided in the standard locust artificial diet [39].

## (g) Statistics

Statistical analyses were performed using the SPSS v.19.0 statistical software (IBM, Armonk, NY, USA). Prior to using parametric analyses, the data normality was confirmed.

### (i) Choice experiment

We used a multivariate analysis of covariance (MANCOVAs) to compare carbohydrate and lipid energy consumption among diets containing different oil sources, with oil sources, diet pairs and sex as independent variables, and final mass as a covariate. There was a significant interactive effect of oil source and diet pair (see §3) on carbohydrate and lipid consumption. Therefore, we ran separate MANCOVAs for each intake target experiment (each lipid source) to determine whether locusts were consistently selecting a specific C:L ratio, regardless of the diet pair, and not eating randomly from between the diet pairs. The insignificant effects of diet pairs (see §3) support the idea that locusts were regulating for a specific C:L ratio. To compare total consumption among the three lipid sources, we used one-way analysis of covariance (ANCOVA) tests, with final body mass as a covariate. Kruskal–Wallis' tests were used to compare the C:L ratios (because these were not normally distributed) among the three-choice experiment (three different lipid sources). One-way ANCOVA tests were used to analyse differences in total energy consumption and mass growth based on sex, with final mass as a covariate.

### (ii) Single-diet experiments

We used a two-way ANOVA to test the effect of diet and sex on development time. To compare lipid content and lipid accumulation, we used a two-way ANCOVA with sex and diet as independent factors, and dry lean body mass as a covariate.

**Table 1.** Summarized details of all statistical tests applied in each experiment.

| goal | test | dependent variables | independent variables | covariate |
|---|---|---|---|---|
| **choice experiments** | | | | |
| comparison of carbohydrate and lipid energy consumption among different oil sources | MANCOVA | carbohydrate and lipid energy consumption | oil source, sex and diet pairs | final body mass |
| to rule out random feeding | MANCOVA | carbohydrate and lipid energy consumption | sex and diet pairs | final body mass |
| self-selected C : I ratio comparison | Kruskal–Wallis | C : I ratio | lipid source | |
| total energy consumption comparison | one-way ANCOVA | energy consumption | lipid source | final body mass |
| mass growth comparison | one-way ANCOVA | mass growth | lipid source | final body mass |
| **single diet experiments** | | | | |
| developmental time comparison | two-way ANOVA | developmental time in days | sex and diet treatments | |
| lipid content comparison | two-way ANCOVA | lipid content | sex and diet treatments | final body mass |
| lipid accumulation comparison | two-way ANCOVA | change in lipid content | sex and diet treatments | final body mass |
| energy consumption comparison | two-way ANCOVA | energy consumption | sex and diet treatments | final body mass |
| oxygen consumption comparison | two-way ANCOVA | oxygen consumption | sex and diet treatments | final body mass |
| carbon dioxide production comparison | two-way ANCOVA | carbon dioxide production | sex and diet treatments | final body mass |
| respiratory exchange ratios comparison | two-way ANCOVA | respiratory exchange ratios | sex and diet treatments | final body mass |

We compared energy consumption, oxygen consumption, carbon dioxide production and respiratory exchange ratios using two-way ANCOVAs, with sex and diet as independent factors and final body mass as a covariate (for a full list of statistical tests see table 1).

## 3. Results

By consuming from both complementary diet pairs (dotted rails in figure 1), both male and female fifth instar nymphs self-selected carbohydrate-to-lipid energy intake ratios close to 2.1C : 1L (figure 1), regardless of lipid source (Kruskal–Wallis: $\chi^2_2 = 3.57$; $p = 0.168$), indicating that nymphs strongly regulate the C : L energy intake ratio. A three-way MANCOVA (see §2) revealed a significant interactive effect of oil source and diet pair (table 2); therefore, we performed separate tests for each choice experiment (table 3). Within each choice experiment with a different oil source, diet pair did not significantly affect carbohydrate and lipid consumption, which supports the idea that locusts were selecting a specific C : L ratio and supports the conclusion that, regardless of the diet pair presented, locusts consumed the same amounts and ratios of carbohydrate and protein (tables 2 and 3). While there was no interactive effect of diet pair and sex, total macronutrient consumption was strongly affected by the sex of the nymphs owing to increased macronutrient consumption by females (figure 1 and table 3). In both sexes, the total energy consumption was higher in locusts that consumed grape seed oil-based diets (ANCOVA: $F_{2,144} = 39.653$; $F_{2,138} = 22.945$; $p < 0.001$ for both sexes, figure 2*a*), but the lowest growth was recorded on this diet treatment (ANOVA: $F_{2,145} = 12.437$; $F_{2,139} = 19.736$ for males and females, respectively; $p < 0.001$ for both, figure 2*b*).

When confined to single diets of either 3P : 4C : 2L or 3P : 6C : 0L, ~93% of locust nymphs successfully moulted to adults in both diet treatments. Of 30 individuals from each sex and diet treatment (120 total), three females died in the 3P : 4C : 2L diet treatment, and two from each of other dietary treatment groups. Development time was not affected by diet or the interaction of diet and sex; however, males developed faster than females (two-way ANOVA, diet: $F_{1,107} = 0.015$; $p = 0.902$; sex: $F_{1,107} = 7.141$; $p = 0.009$; diet × sex: $F_{1,107} = 0.015$; $p = 0.902$; figure 3*a*). The lipid contents of the freshly moulted adults and lipid storage growth were not affected by the diet, sex or the interaction between them (lipid content, two-way ANCOVA: diet: $F_{1,106} = 1.841$; $p = 0.178$; sex: $F_{1,106} = 1.614$; $p = 0.207$; diet × sex: $F_{1,106} = 0.758$; $p = 0.386$; figure 3*b*) (lipid storage growth, two-way ANCOVA: diet: $F_{1,106} = 2.412$; $p = 0.237$; sex: $F_{1,106} = 0.094$; $p = 0.760$; diet × sex: $F_{1,106} = 0.560$; $p = 0.456$; figure 3*c*). However, regardless of sex, nymphs that were reared on the carbohydrate-based diet (3P:6C:0L) showed a higher daily energy consumption rate (two-way ANCOVA, diet: $F_{1,106} = 5.809$; $p = 0.018$; sex: $F_{1,106} = 0.166$; $p = 0.684$; diet × sex: $F_{1,106} = 0.351$; $p = 0.555$; figure 3*d*).

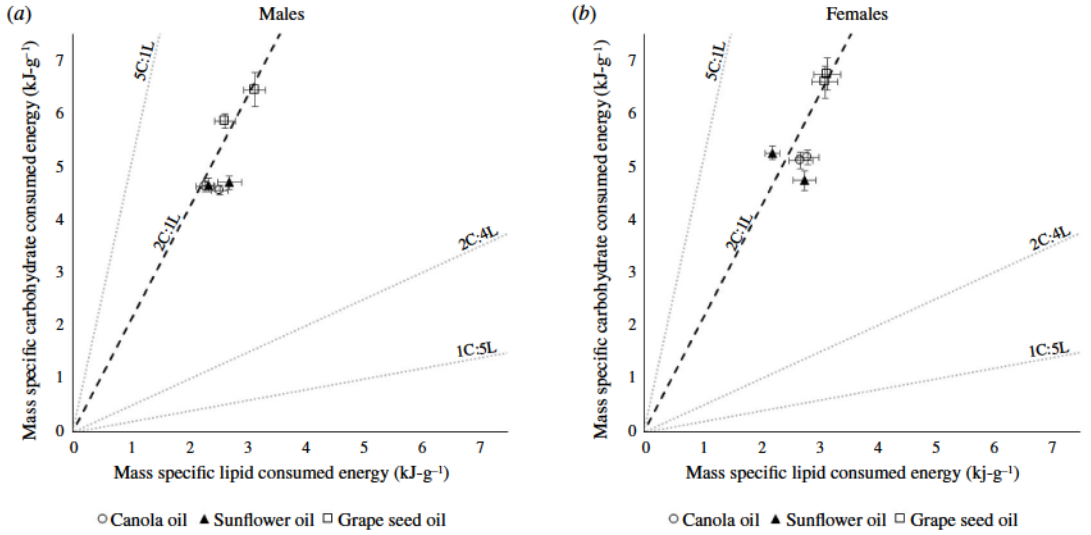


**Figure 1.** *Locusta migratoria* fifth instar male (*a*) and female (*b*) nymphs self-select a narrow range of carbohydrate-to-lipid energy ratio, regardless of lipid source (represented by different symbols). The dotted rails represent the different diets, which were used as pairs for choice experiments (see §2). The dashed lines represent the average self-selected intake target ratio. The error bars represent ± s.e.m. For sample size, see figure 2*a*.

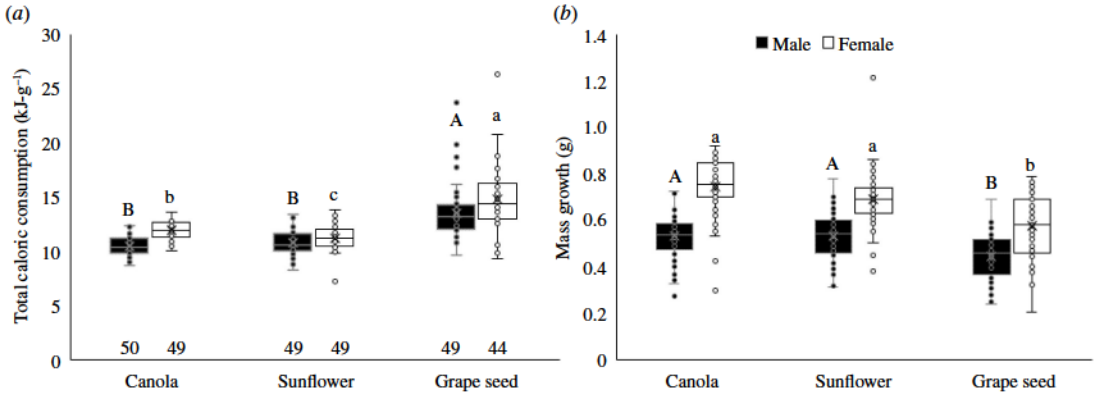


**Figure 2.** Nymphs reared on diets that contained lipids from grape seeds consumed more energy (*a*) but had lowest mass growth (*b*) during the fifth instar, for both males (black boxes) and females (white boxes). The names on the *x*-axis represent the sources of the lipids that were used for each diet group. Groups with similar letters did not differ significantly (Bonferroni *post hoc* tests, $p < 0.05$). The capital letters refer to comparisons among males and lowercase for comparisons among females. In this figure and the following figures, and interquartile ranges and medians are represented by the boxes and centre lines, respectively, with an X to indicate the mean, and the numbers inside panel (*a*) indicate the number (sample size) of individuals in each treatment group.

**Table 2.** Multiple analysis of covariance (MANCOVA) of all three choice experiments, combined.

| effect | *F*-value | *p*-value | Wilks' Λ |
|---|---|---|---|
| oil source | $F_{2,277} = 26.427$ | <0.001 | 0.704 |
| diet pair | $F_{1,277} = 0.445$ | 0.812 | 0.994 |
| sex | $F_{1,277} = 47.18$ | <0.001 | 0.745 |
| oil source × diet pair | $F_{2,277} = 2.658$ | 0.032 | 0.963 |
| oil source × sex | $F_{2,277} = 2.298$ | 0.058 | 0.968 |
| diet pair × sex | $F_{1,277} = 0.629$ | 0.534 | 0.995 |
| oil source × diet pair × sex | $F_{2,277} = 1.017$ | 0.398 | 0.985 |

*Notes:* Carbohydrate and lipid consumption are dependent variables, whereas oil source, sex and diet pairs are independent variables, with final body as a covariate.

Dietary lipid content affected locust metabolism. Nymphs that were reared on the carbohydrate-only diet had higher oxygen consumption rates (figure 4*a*) and carbon dioxide production rates (figure 4*b*) than those on diets with lipids. While there was no effect of sex nor a significant interactive effects of sex and diet on oxygen consumption rate (two-way ANCOVA, diet: $F_{1,75} = 10.948$, $p < 0.001$; sex: $F_{1,75} = 3.408$, $p = 0.069$; diet × sex: $F_{1,75} = 0.037$, $p = 0.849$), the carbon dioxide production rate was higher in females than males (two-way ANCOVA, diet: $F_{1,75} = 24.191$, $p < 0.001$; sex: $F_{1,75} = 4.923$, $p = 0.030$; diet × sex: $F_{1,75} = 0.053$, $p = 0.818$). The respiratory exchange ratio (RER; carbon dioxide production divided by oxygen consumption) was higher in

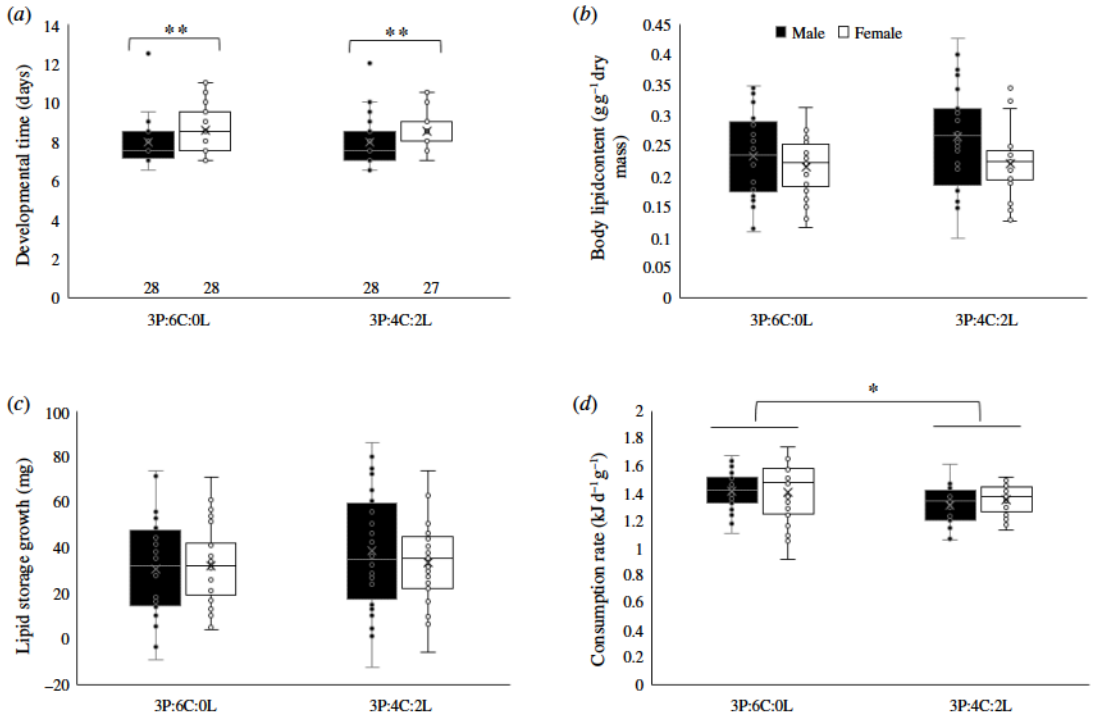


**Figure 3.** When confined to no-choice diets, nymph did not show any effect of diets on developmental time (*a*), final lipid content (*b*) or lipid growth (*c*), for both males (black boxes) and females (white boxes). However, nymphs that reared on carbohydrate-based diets exhibited a higher energy consumption rate than nymphs reared on lipid-based diets (*d*). In this and the following figure, the ratios on the *x*-axis represent the diet's caloric ratio among proteins (P): carbohydrates (C): lipids (L).

**Table 3.** Multiple analysis of covariance (MANCOVA) of three different choice experiments using three different lipid sources.

| lipid source | effect | *F*-value | *p*-value | Wilks' Λ |
|---|---|---|---|---|
| canola oil | diet pair | $F_{11,93} = 0.779$ | 0.462 | 0.984 |
| | sex | $F_{11,93} = 28.078$ | <0.001 | 0.624 |
| | diet pair X sex | $F_{11,93} = 0.202$ | 0.818 | 0.996 |
| sunflower oil | diet pair | $F_{11,92} = 4.227$ | 0.018 | 0.916 |
| | sex | $F_{11,92} = 20.393$ | <0.001 | 0.693 |
| | diet pair × sex | $F_{11,92} = 1.037$ | 0.359 | 0.978 |
| grape seed oil | diet pair | $F_{11,87} = 0.839$ | 0.436 | 0.981 |
| | sex | $F_{11,87} = 10.948$ | <0.001 | 0.799 |
| | diet pair × sex | $F_{11,87} = 0.815$ | 0.815 | 0.982 |

*Notes:* Carbohydrate and lipid consumption are dependent variables, and whereas sex and diet pairs are independent variables, with final body as a covariate. Non-significant diet effect confirms the non-random diet selection.

nymphs that ate the carbohydrate-only diet compared with the diet that also contained lipid (ANCOVA, diet: $F_{1,76} = 31.126$, $p < 0.001$) (figure 4*c*). Females had higher RERs than males (sex, $F_{1,76} = 3.982$, $p = 0.049$), but there was no interactive effect of sex and diet on RER (diet*sex, $F_{1,76} = 0.561$, $p = 0.456$) (figure 4*c*). For locusts consuming diets without lipids, the RERs exceeded 1.1, indicating a net high rate of de novo lipogenesis [23,24], whereas nymphs consuming diets with the preferred carbohydrate-to-lipid ratio had an average RER of 0.97 (figure 4*c*).

## 4. Discussion

According to most optimal foraging theory models, consumers are expected to optimize foraging by maximizing net energy intake rate ((energy intake–energy use)/time; [40,41]). The energy costs are commonly thought of as the costs of locomotion during foraging and food handling [40]. The metabolic costs of post-consumption assimilation are rarely considered. Here, we demonstrate that the costs of energy storage accumulation are significantly reduced when some of the energy consumed derives from lipids rather than purely from carbohydrates, providing at least a partial explanation for our finding that locusts preferentially consume some lipids when available. Similarly, it has been demonstrated that post-hibernating carnivorous beetles had lower heat production rates following lipid-rich meals than when consuming carbohydrate- or protein-rich meals,

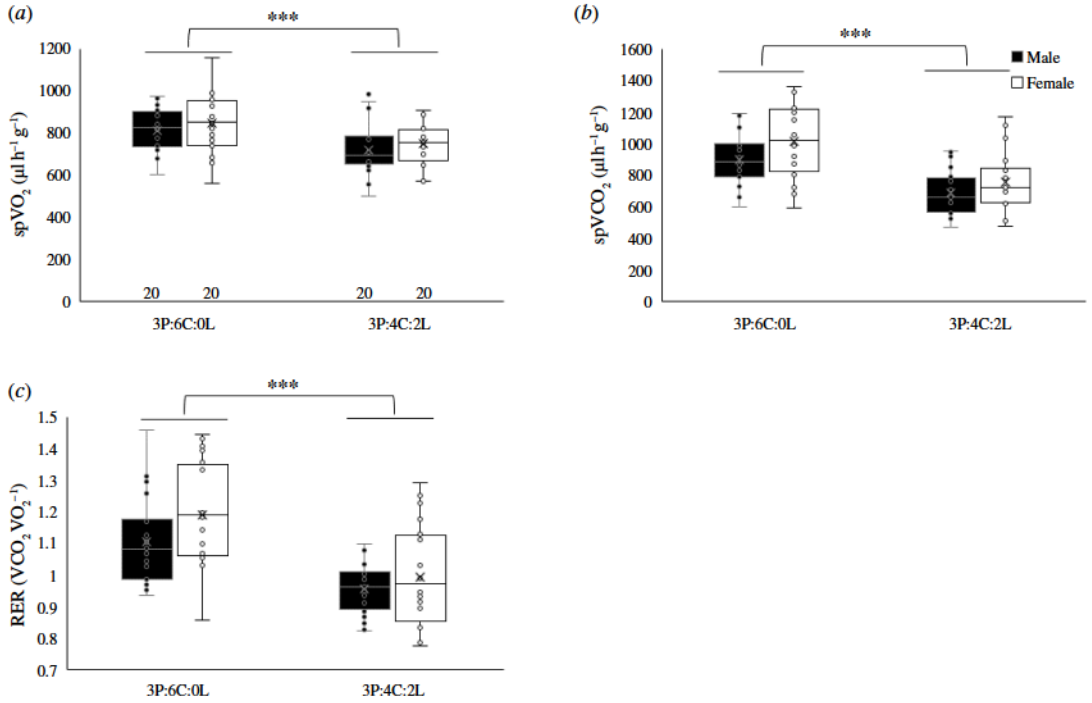


**Figure 4.** Nymphs fed the carbohydrate-based diets exhibited higher oxygen consumption (*a*), $CO_2$ production (*b*) and higher RER that exceeded 1 (*c*).

when restoring fat stores [42]. Because the metabolic pathways for conversion of carbohydrate to lipid are strongly conserved, the higher accumulation efficiency associated with lipid relative to carbohydrate consumption is likely to have general adaptive benefits for animals broadly. For example, for animals needing to accumulate lipids, all else being equal, replacing some dietary carbohydrate with lipid would likely decrease necessary foraging time and free up time for other purposes such as mating and/or decrease vulnerability to predation.

While it was well documented that omnivorous and carnivorous animals from invertebrates to primates regulate protein-to-NP caloric ratios (including both carbohydrates and lipids) [13,43–47], little has been known about the capacity of herbivores to sense and regulate lipid intake (but see [48]). We showed that locusts consistently foraged for a 2.1C : 1L energy ratio, regardless of lipid source and sex, clearly demonstrating a capacity to distinguish lipids from carbohydrates and other nutrients (figure 1). We used three different vegetable oils to control for different tastes potentially eliciting different total consumption rates. Locusts reared on the grape seed oil-based diets had the highest total energy consumption but the lowest mass growth (figure 2). Since the composition of the major fatty acids in the three oils is relatively similar [35,36], this finding may be explained by higher secondary metabolite content in grape seeds [49], which might require the nymphs to invest significant energy for detoxification [50]. Females consumed more than males in all three choice experiments, consistent with their higher growth rates, but regulated to the same C : L energy ratio as males. This indicates that the tight intake regulation for a specific C : L energy ratio in both sexes likely benefits their developmental performance and fitness similarly.

This is the first study in any animal to demonstrate that replacing some carbohydrates in the diet with lipids improves energy storage accumulation efficiency. Locusts fed diets without lipids consumed more diet (by both mass and energy) to achieve similar total body lipid contents and lipid growth to locusts provided diets with lipid (figure 3). In addition, locust nymphs reared on zero-lipid diets had higher metabolic rates than locusts consuming diets with the preferred carbohydrate-to-lipid ratio (figure 4). The high RER, which was above 1.1 for the locusts on the zero-lipid diets is consistent with the conclusion that these locusts were synthesizing lipids de novo from carbohydrates (figure 4) [23,24].

Our dataset, with some assumptions, gave us an opportunity to estimate the inefficiency of synthesizing and storing lipids from consumed carbohydrates compared with storing lipids from consumed lipids. First, the two diets had the same protein content (~33%), and the RERs for locusts eating either diet were well above the 0.85 value associated with protein catabolism. Therefore, it is likely that there were no differences in the use of dietary protein for metabolism between the two treatments because protein catabolism was likely minimal for many locusts. Second, locust glycogen energy stores are very low, stable and limited in growth compared with lipid (~22 times less than lipid, 154 and 3350 J $g^{-1}$, respectively: Talal *et al.* [51]); therefore, we made our calculations assuming that the energy costs of glycogen anabolism are negligible compared with lipid anabolism for ingested carbohydrates. Third, the locusts fed diets without lipids had metabolic rates that were 0.063 kJ $d^{-1}$ $g^{-1}$ higher than locusts fed diets with lipids, and on both diets, the energy accumulation in body lipid stores was 0.16 kJ $d^{-1}$ $g^{-1}$. Using equation (4.1), we calculated the energy efficiency of converting carbohydrates to lipid as 72%, meaning that 28% of the energy of lipid-converted carbohydrate was lost during de novo lipogenesis, very similar to estimates for rodents [16].

$$\text{efficiency} = 100\%\left(1 - \frac{\text{extra energy expenditure of carbohydrate based treatment group}}{\text{extra energy expenditure of carbohydrate based treatment group} + \text{daily lipid energy deposition}}\right). \quad (4.1)$$

Our finding that locusts have an intake target for C : L and that lipid intake can improve lipid storage accumulation efficiency sets the stage for many further investigations. Mechanistically, what are the sensory pathways involved in regulating C : L

intake? Ecologically, do individuals in locust outbreaks search out foods that are high in lipids, or does the availability of dietary lipids support population outbreaks? Comparatively, do migratory species such as locusts that accumulate large amounts of lipid have lower C : L targets? The realization that differential costs of lipid and carbohydrate assimilation into different uses can significantly affect animal energy balance opens a new area of nutritional ecophysiology that complements and advances both optimal foraging theory and the geometric framework for nutrition.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. The raw data are stored in Dryad [52].

Supplementary material is available online [53].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. S.T.: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing—original draft, writing—review and editing; A.C.: investigation, methodology, writing—original draft, writing—review and editing; G.M.O.: investigation, methodology, writing—original draft, writing—review and editing, J.B.: investigation, methodology, writing—original draft, writing—review and editing, J.F.H.: conceptualization, investigation, methodology, project administration, resources, supervision, writing—original draft, writing—review and editing; A.J.C.: conceptualization, funding acquisition, investigation, methodology, project administration, resources, supervision, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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