Research

Author for correspondence:
Stav Talal
e-mail: stalal@asu.edu

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Plant carbohydrate content limits performance and lipid accumulation of an outbreaking herbivore

Stav Talal[1,2], Arianne J. Cease[1,2], Jacob P. Youngblood[1], Ruth Farington[1], Eduardo V. Trumper[3], Hector E. Medina[4], Julio E. Rojas[5], A. Fernando Copa[6] and Jon F. Harrison[1]

[1]School of Life Sciences, Arizona State University, 427 E Tyler Mall, Tempe, AZ 85281, USA
[2]School of Sustainability, Arizona State University, Tempe, AZ, USA
[3]Instituto Nacinal de Tecnología Agropecuaria, Manfredi, Argentina
[4]Dirección de Sanidad Vegetal - SENASA, Argentina
[5]Departamento de Campañas Fitosanitarias, Dirección de Protección Vegetal, SENAVE, Paraguay
[6]Universidad Autónoma Gabriel René Moreno, Santa Cruz, Bolivia

ST, 0000-0003-1181-5291; AJC, 0000-0002-4778-0775; JFH, 0000-0001-5223-216X

Locusts are major intermittent threats to food security and the ecological factors determining where and when these occur remain poorly understood. For many herbivores, obtaining adequate protein from plants is a key challenge. We tested how the dietary protein : non-structural carbohydrate ratio (p : c) affects the developmental and physiological performance of 4th-5th instar nymphs of the South American locust, *Schistocerca cancellata*, which has recently resurged in Argentina, Bolivia and Paraguay. Field marching locusts preferred to feed on high carbohydrate foods. Field-collected juveniles transferred to the laboratory selected artificial diets or local plants with low p : c. On single artificial diets, survival rate increased as foods became more carbohydrate-biased. On single local plants, growth only occurred on the plant with the lowest p : c. Most local plants had p : c ratios substantially higher than optimal, demonstrating that field marching locusts must search for adequate carbohydrate or their survival and growth will be carbohydrate-limited. Total body lipids increased as dietary p : c decreased on both artificial and plant diets, and the low lipid contents of field-collected nymphs suggest that obtaining adequate carbohydrate may pose a strong limitation on migration for *S. cancellata*. Anthropogenic influences such as conversions of forests to pastures, may increase carbohydrate availability and promote outbreaks and migration of some locusts.

1. Introduction

Many invertebrates exhibit intermittent extreme population outbreaks with major effects on ecosystems and agriculture, yet the ecological factors determining where and when such outbreaks occur remain poorly understood [1,2]. For outbreaking insects, the protein : non-structural carbohydrate macronutrient ratio (p : c), specific essential amino acids, phosphorus, sodium, sterols and plant secondary compounds have all been suggested as potential limiting factors [3]. The hypothesis that weather-driven effects on plant stress promote insect outbreaks has very mixed support, suggesting that understanding diet quality effects on insects requires consideration of life history, clade and the environmental context [3–5]. Also, the specific nutrients limiting herbivores can vary with life-stage, past dietary history [6] and population location [7,8]. Thus, understanding the role of diet quality in insect outbreaks requires assessment of diet quality effects in a specific field context. In this research, we tested the how dietary p : c ratio affects the developmental and physiological performance of field-collected 4th-5th instar nymphs from an outbreaking population of

the South American locust, *Schistocerca cancellata*, and assess their p : c requirements relative to the p : c of the common local plants.

Despite the long history of nutritional research on locusts, the role of dietary quality in population cycles remains unclear (reviewed in [9,10]). Over thousands of years, locust outbreaks correlate with weather events, supporting potential roles for weather in determining survival, egg success and possibly diet quality [11]. Shortages of foods with required macronutrient ratios have been linked to clumping behaviour that initiates gregarization, as well as migratory behaviour [12–14]. While classically, insect herbivores were thought to most commonly be limited by protein availability [15], a few recent studies of locusts in the field have suggested that plants with relatively low p : c are necessary for success of outbreaks. In inner Mongolia, growth, survival and migratory capacity of *Oedaleus asiaticus* is enhanced by high-carbohydrate artificial and natural diets; moreover, locusts in the field prefer high-carbohydrate plants, and grazing-induced decreases in plant p : c increase local locust abundance [16,17]. Similarly, local population density of the Australian plague locust, *Chortoicetes terminifera*, has been shown to be negatively correlated with plant protein content [18]. However, clear linkages between plant p : c and field performance are lacking for *C. terminifera*. Field populations of *C. terminifera* with higher haemolymph protein levels had better immune function, and body lipid contents varied little across field sites, providing support for positive effects of higher protein availability for this locust [19]. The Senegalese locust, *Oedaleus senegalensis*, grows and develops fastest, and survives at higher rates on artificial diets with a low p : c, and are more common on fields with low p : c [20,21]. However, it is unknown whether the p : c of local plants affects performance of *O. senegalensis*. Findings from artificial diet studies may not match findings from local plant studies because herbivore performance is affected by many plant parameters including leaf toughness and concentrations of plant secondary compounds. Clearly, additional nutritional ecological studies of locusts in the field are needed to develop predictive models linking plant macronutrient ratios to locust outbreaks.

In this study, we took advantage of an ongoing 5-year expansive population upsurge of the South American locust, *S. cancellata*, to study their nutritional ecology and physiology in the field. To understand the linkages between plant macronutrient content and locust performance in their natural habitat, we measured the macronutrient levels in the most common local plants, tested performance (growth, development rate, survival, lipid accumulation and migratory capacity based on lipid stores) for locust nymphs eating artificial and local plant diets, as well as performing measures of macronutrient preference in the laboratory and field. These studies clearly show that *S. cancellata* late-instar nymphs are carbohydrate-limited in the field, with strong negative effects on survival and the lipid stores needed to migrate.

2. Methods

(a) Animals, field collections and experimental design
Schistocerca cancellata was a very destructive agricultural pest in the late 1800s to mid-1900s in South America, with a limited narrow permanent breeding zone in western Argentina, and occasional massive outbreaks covering up to 4 000 000 km^2 and six countries

[22]. Populations of *S. cancellata* in South America declined considerably from the 1950s through to 2015, partially owing to eradication efforts based mostly on the use of synthetic pesticides [23,24]. However, beginning in 2015, continuing to today (May 2020), national-emergency-triggering outbreaks of *S. cancellata* have occurred in Argentina, Bolivia and Paraguay [22,25].

Outbreaks of *S. cancellata* in Paraguay were tracked by Servicio National de Calidad y Sanidad Vegetal y Semillas (SENAVE), and found along roads in typical edge habitat of the Chaco dry subtropical zone (April 2019). Animals were captured by netting, placed in screened metal containers and fed local vegetation, primarily grasses (mostly *Paspalum* sp.). Collections contained predominantly fourth instar nymphs.

To assess the availability of protein and carbohydrate in the field, we collected samples of many of the predominant plant species in locations where locusts were observed, including all plants that were observed to be fed on by locusts. Samples were dried and transported to the laboratory at ASU (Arizona State University) where the percentage of non-structural carbohydrate, carbon and nitrogen were measured.

We conducted all experiments at the SENAVE regional office. Our goal was to determine the preferred macronutrient ratio and the effect of diets on growth performance of *S. cancellata* nymphs that were collected from the field. Therefore, we initiated experiments 2–3 days after collection. At the SENAVE regional office, experiments took place in a room maintained near the natural light : dark cycle (approx. 12 L : 12 D). Temperature and relative humidity in the room were measured with a Hobo Onset logger. Temperatures and relative humidity in the room averaged 32.2 ± 1.94°C, 58.7 ± 4.57% (mean ± s.d.).

(b) Artificial diets
The artificial diets were made in our Arizona State University (ASU) laboratory (described in [26]). We used eight different isocaloric artificial foods in different assays which varied in protein and digestible carbohydrates: 0p : 42c, 7p : 35c, 14p : 28c, 21p : 21c, 28p : 14c, 35p : 7c, 42p : 0c and 0p : 0c (% protein and % digestible carbohydrates, by dry mass). All the diets contained 54% cellulose and 4% of vitamins and salts, with the exception of the 0 : 0 diet, which contained an extra 42% cellulose in place of the macronutrients. The proteins were provided as a mix of 3 : 1 : 1 casein : peptone : albumen. The carbohydrate was provided as a 1 : 1 mix of sucrose and dextrin.

(c) Field tests of hunger for high-carbohydrate versus high-protein food
The field tests of hunger for high-protein versus high-carbohydrate food were conducted in three locations, along roads where marching was observed. We placed four Petri dishes (approx. 9 cm diameter) in locations that we anticipated would be encountered by locusts. These contained 42p : 0c, 0p : 42c, 21p : 21c and 0p : 0c (as described in [27], see the electronic supplementary material, S1 movie). These dishes were observed from approximately 7 to 10 m distance for 30–60 min, because these marching locust bands were occasionally interrupted by passing vehicles. We counted locusts as feeding from the dish if an individual spent at least 30 s in the dish, enough time to taste the food and decide whether to feed.

(d) Protein : carbohydrate intake target assessment with artificial diets
Intake targets were measured for 6 days with two different diet pairs. Twenty-four male and 24 female fourth instar nymphs were placed individually in locust containers (plastic boxes 11 × 16 × 4 cm, with holes drilled in the roof for ventilation). Each container contained two complementary food dishes, one with a high p : c and one with a low p : c, and one water tube.

Half of the locusts were provided with the choice between 35p : 7c and 7p : 35c diets, while the other half were provided with the choice between 28p : 14c and 7p : 35c diet. The locust and dry food were weighed at the start of the experiment. Every 3 days, the locusts were weighed, and their food dishes refreshed with new pre-weighed food dishes. Remaining food was dried to constant mass over silica gel beads (approx. 48 h), then weighed. We calculated protein and carbohydrate consumption by multiplying the total dry mass of each food by the percentage of each macronutrient and summing the total amount of each nutrient eaten by a given locust over the duration of the experiment.

(e) Plant choice (buffet) experiment

We tested locust preference for seven different abundant local plants with a buffet experiment using 10 fourth and fifth instar nymphs from both sexes. With help from plant taxonomists, we identified six of the plants to the genus level (*Paspalum* sp*., Celtis* sp*., Mikania* sp*., Grabowskia* sp*., Prosopis* sp. and *Digitaria* sp.) and one to the subfamily level (Celastraceae). Each pre-weighed individual was provided with a known fresh mass (approx. 0.8 g) of each of seven plants within a plastic locust container. Five separate samples of each fresh plant were dried to constant weight to allow estimation of the dry mass and water content of each plant that was provided. After 2 days, each locust was re-weighed, and the plants in each cage were separated and dried to constant weight in an oven (approx. 60°C). The dry mass consumption of each locust of each plant type was calculated from the difference between the estimated initial dry mass and the ending dry mass for that plant species. Dried samples of each plant were taken to ASU for macronutrient chemical and elemental analyses.

(f) The effect of protein : non-structural carbohydrate ratio of artificial diets on performance

Consumption and weight gain on artificial diets were measured over 8 days. Locusts were provided with one of five single artificial diets: 35p : 7c, 28p : 14c, 21p : 21c, 14p : 28c or 7p : 35c, and a water tube. Ten male and 10 female fourth instar nymphs were assessed for each diet treatment ($n = 100$ locusts). The locusts and dry food were weighed at the start and end of the experiment, with the remaining food dried to a constant mass before weighing. The specific macronutrient consumptions were calculated as explained above. We also observed the animals daily and recorded moulting and deaths. Additionally, we measured rates of development, mass growth and survival (but not consumption owing to logistical constraints) for an additional five male and five female locusts on each diet.

(g) The effect of protein : non-structural carbohydrate ratio of local plants on survival, development and mass growth

We measured weight gain, development and survival (not consumption) over 6 days for *S. cancellata* kept individually in locust containers with a water tube and ad libitum amounts of one of seven different plants collected locally (the same plants used in the plant choice experiment). Each plant treatment group had 10 male and 10 female fourth instar nymphs ($n = 140$ locusts). Locusts were weighed at the start and end of the experiment. We also observed the animals daily and recorded moulting and deaths.

(h) Effect of diet on total body lipid content

We measured body lipid content of locusts from the experiments measuring the effect of artificial and plant diets on growth, as well as for fourth instar locusts collected directly from marching bands. At the end of the experiments, or immediately after field

collection, we froze the locusts and stored them in ethanol for transport to ASU. We then analysed total body lipid using a chloroform extraction technique [28].

(i) Carbohydrate, carbon and nitrogen composition of local plants

We measured macronutrient contents of leaves for all plants used in the intake (buffet) and performance assays and for all field plants on which locusts were observed to feed. In order to prepare the dried plant leaf samples for the chemical analysis, we ground them (30 s, 30 Hz) using a Retsch MM 400 ball mill (Haan, Germany). We measured plant non-structural carbohydrate content using the phenol-sulfuric acid method [29]. In order to measure the total carbon (C) and nitrogen (N) content in each plant, approximately 2.5 µg dry plant samples (one measurement per field surveyed plant and two for plants from experiments), weighed using a micro-balance (MSA6.6S0TRDM, Sartorius Mechatronics Corporation, Tempe, AZ, USA) were analysed with a CHN PE 2400 elemental analyzer (Perkin Elmer, Waltham, MA, USA) at the Goldwater Environmental Laboratory, an ASU core facility. In order to estimate crude protein content, we multiplied plant N content by 6.25 [30].

(j) Statistics

Statistical analyses were performed using SPSS 20.0 (IBM). Prior to using parametric analysis the data normality was confirmed. For the intake target experiments, to rule out random feeding on different diet pairs, we employed multiple analysis of covariance (MANCOVA), using diet pairs and sex as different blocks and final body masses as covariates. To compare the total consumption among no-choice diet treatments, we used two-way ANCOVAs with diet treatment and sex as blocks, and body mass as a covariate. We used a binary logistic regression model to test whether the macronutrient content in locust artificial diets affected their survival. To compare responses of nymphs eating the different local plants, we used one-way ANOVAs to test effects on body mass and growth rate. To test the effects of no-choice experiments (artificial diets) on body lipid content, we used ANCOVAs with dry mass after lipid extraction as a covariate.

3. Results

(a) Macronutrient choice of marching *Schistocerca cancellata* nymphs and macronutrient contents of local plants

In general, our results showed that marching *S. cancellata* preferred carbohydrate-biased food, while local plants were protein-biased. In all cases, the march flowed over the four dishes of food and many individuals encountered the dishes. For all three trials, many more locusts visited the 0p : 42c dish than the other dishes, and the distribution of locusts visiting the dishes was significantly different from random in all cases (figure 1a and the statistics within). By contrast, most plants at the sites where we found large bands of locusts had p : c ratios greater than 1 (figure 1b) (see the electronic supplementary material, table S1 for plant raw data).

(b) Intake targets and performance on artificial diets

Both males and females had an intake target of approximately 1 : 2 p : c (electronic supplementary material, figure S1, open symbols). There were no effects of sex, diet treatment pairs or the interaction between sex and treatment diets on the
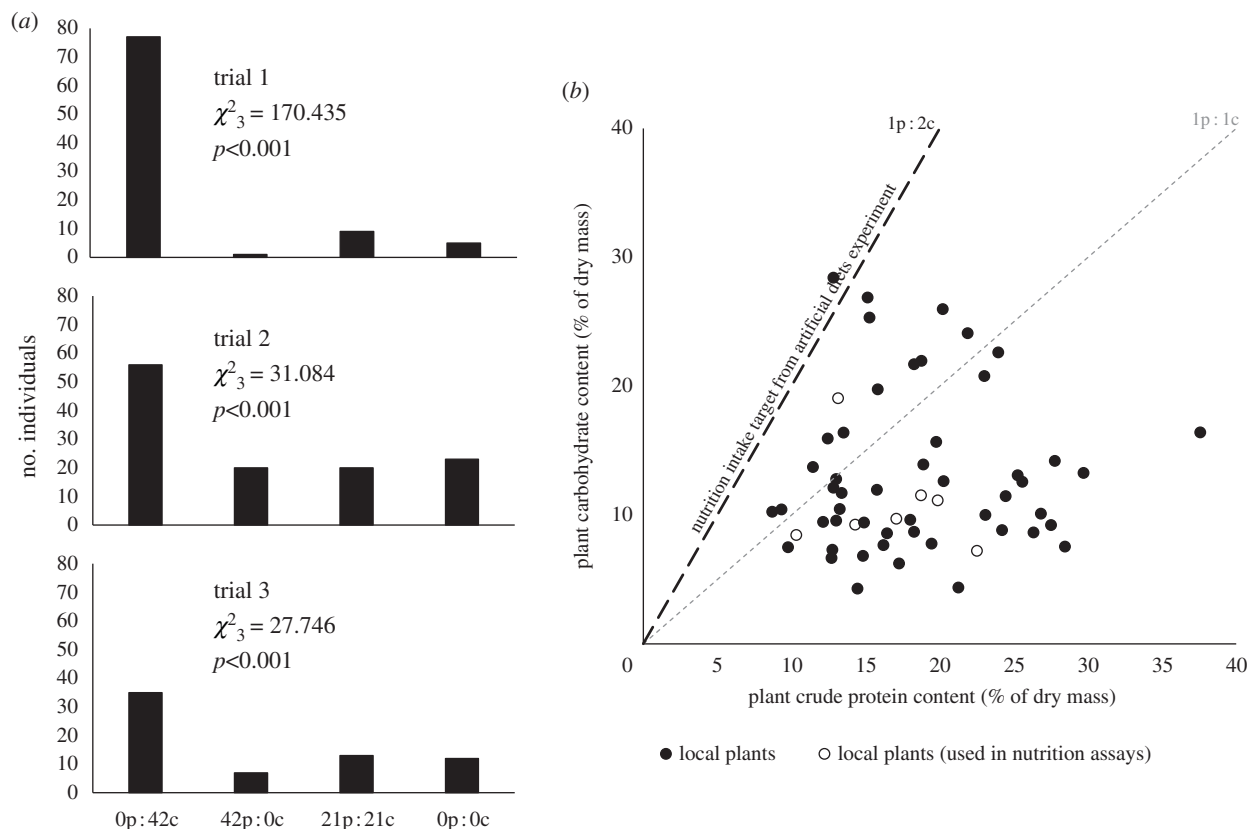
Figure 1. (a) Marching *Schistocerca cancellata* preferred to feed on diets high in carbohydrate. The *x*-axis indicates the per cent protein to non-structural carbohydrate ratio (p : c) of the dry artificial diets. The *y*-axis indicates the number of locusts observed feeding in the bowls over 30–60 min during three different trials. In all cases, χ^2 analysis indicated that locusts fed non-randomly. (b) Protein and carbohydrate contents of the most abundant plants where marching locusts were found, compared to the preferred intake target (from artificial diet intake target experiment) of *S. cancellata* (0.5 p : c, black dashed line) and a p : c of 1 (grey dashed line). Open circles represent local plants that were fed to locusts in plant choice and performance assays.

amount of protein and carbohydrates eaten (electronic supplementary material, figure S1, and the statistics within). The intake target of field-collected nymphs was more carbohydrate-biased compared to the majority of the most common local plants (figure 1b).

Dietary p : c did not affect the total amount eaten (electronic supplementary material, figure S1, filled symbols, see the statistics within). Higher dietary p : c was associated with a lower percentage of surviving nymphs (figure 2a). The survival rates were not affected by sex of the locusts. Nymphs reared on the most carbohydrate-biased diets had the lowest body masses, but otherwise, body mass was not affected by diet or sex (figure 2b). Developmental rate (time to fifth instar) was not significantly affected by artificial diet for either sex (electronic supplementary material, figure S2).

(c) Performance and diet preference of *Schistocerca cancellata* nymphs on local plants

There were no statistically significant differences in survival among the groups feeding on different local plants (electronic supplementary material, figure S3). However, feeding on different plants for 6 days affected nymphal wet mass, with the nymphs fed on *Paspalum* sp. being the heaviest (figure 2c) and the only group to gain mass during the experiment. In fact, on average, nymphs reared on local plants other than *Paspalum* sp., lost weight (figure 2d). The fourth and fifth instar nymphs preferred to eat *Paspalum* sp. in the buffet experiment in which they had the choice of seven local plants (electronic supplementary material, figure S4).

Nymphal relative consumption in the buffet experiment was positively correlated with plant carbohydrate content (figure 3a), and plant C : N ratio (figure 3b).

(d) The effect of dietary macronutrients on body lipid content

Total nymphal lipid content increased with the carbohydrate concentration in their ingested artificial diet (figure 4a). The nymphs from the intake target experiment (choice) had the same lipid content as the two most carbohydrate-biased artificial diet treatments (no choice). Locusts fed artificial diets had higher lipid content than locusts fed local plants, or marching locusts collected directly from the field (electronic supplementary material, figure S5). Total lipid content of plant-reared nymphs increased with the plant C : N content (figure 4b).

(e) A comparison of *Schistocerca cancellata* nymphs reared on artificial diets to plant-fed nymphs

Locusts performed better on artificial diets. The average survival on artificial diets was almost twice that than on local plant diets (84.7% versus 49.3%, $\chi^2_1 = 13.41$, $p < 0.001$). Despite the same initial mass (0.448 ± 0.009 g, 0.453 ± 0.012 g, *t*-test, $t_{194} = 0.301$, $p = 0.763$), the artificial diet-reared locusts were approximately 37% heavier than plant-fed locusts after 6 days of diet trails (0.575 ± 0.016 g versus 0.419 ± 0.016 g, $t_{194} = 6.276$, $p < 0.001$). The fourth-instar nymphs that were collected directly from marching bands in the field, showed similar
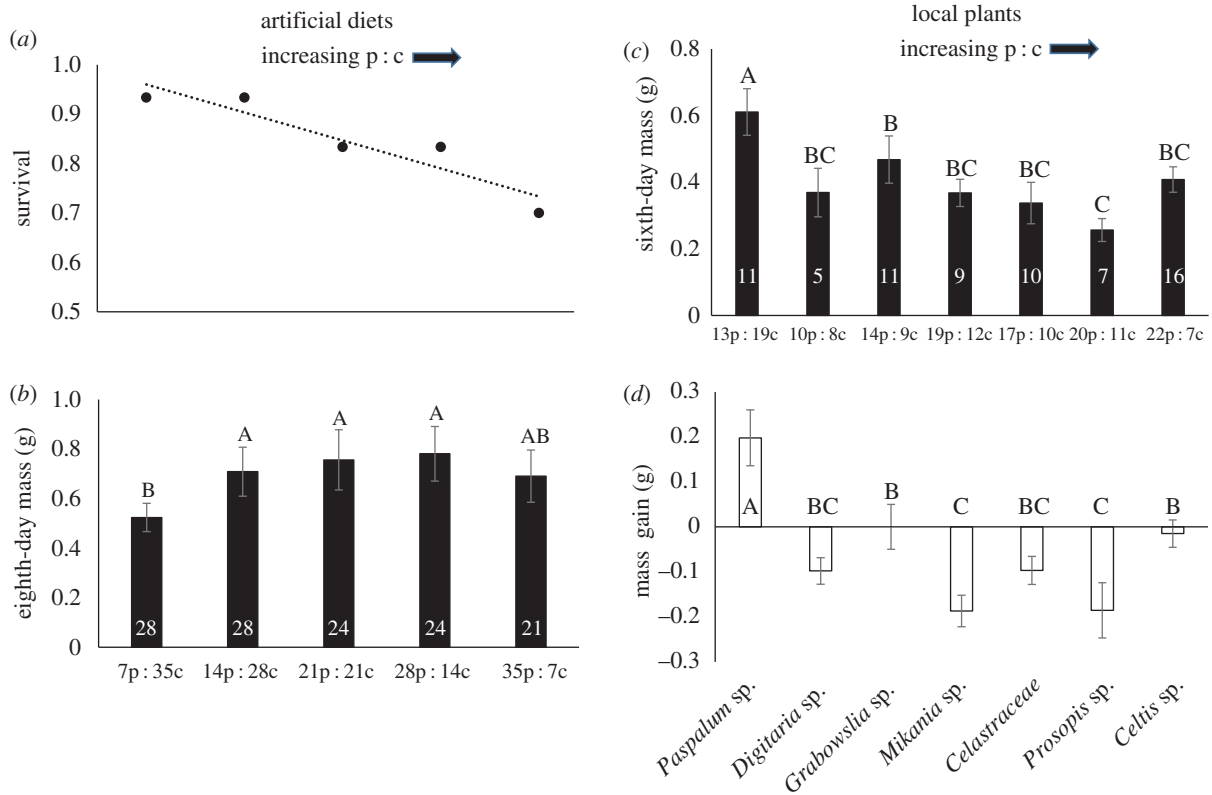
Figure 2. Performance of *S. cancellata* nymphs consuming single artificial diets or a single local plant. (*a*) Survival declined as dietary p : c increased on the artificial diets (binary logistic regression model: $\chi_2^2 = 7.033$, $p = 0.03$; p : c ratio slope $= 0.316$, $p = 0.007$). Sex did not affect survival (sex slope $= -0.108$, $p = 0.816$). (*b*) Macronutrient ratio in artificial diets but not sex significantly affected body masses (two-way ANCOVA: diet effect: $F_{9,117} = 6.323$, $p < 0.001$; sex effect: $F_{1,117} = 1.511$, $p = 0.221$; diet $\times$ sex effect: $F_{4,117} = 1.346$, $p = 0.257$; initial wet mass used as a covariate). (*c*) Body masses differed significantly between different plant fed locusts (ANOVA, $F_{6,62} = 12.357$, $p < 0.001$). (*d*) Mass gain was only positive on *Paspalum* sp. (ANOVA, $F_{6,62} = 33.0$, $p < 0.001$). In this and subsequent figures, letters indicate significant difference (Bonferroni *post hoc* tests, $p < 0.05$), and the number inside the black bars indicate the number of individuals in each treatment group.
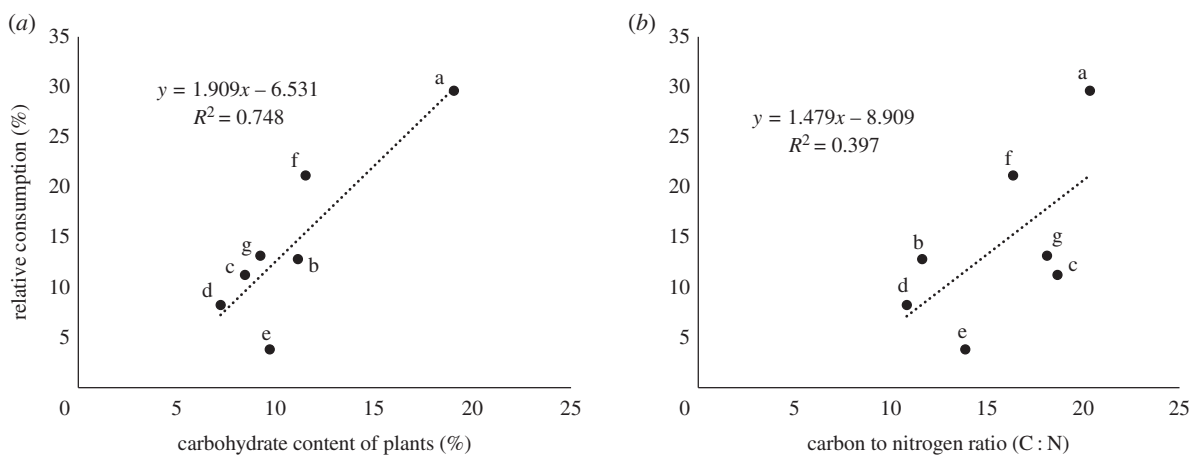

Figure 3. *Schistocerca cancellata* nymphs preferred local plants that were high in carbohydrate. During the buffet experiment, *S. cancellata* consumed more of plants with higher carbohydrate content (*a*), or plants with a higher carbon to nitrogen ratio (*b*). The small letters near each data point represent a plant which was used in experiments: a, *Paspalum* sp.; b, *Prosopis* sp.; c, *Digitaria* sp.; d, *Celtis* sp.; e, Celastraceae; f, *Mikania* sp.; and g, *Grabowslia* sp.

total body lipid content to locusts reared on local plants, and four-times lower total body lipids as locusts reared on artificial diets (electronic supplementary material, figure S5).

4. Discussion

This is the first study, to our knowledge, to study the nutritional ecology of free-living marching locust bands in their natural habitat, and we found that *S. cancellata* nymphs developing in

the Gran Chaco forest of Paraguay seek and prefer high carbohydrate (low p : c) plants. Nymphs preferred artificial diets with a p : c of 1 : 2 and consumed local plants in proportion to their carbohydrate content; these feeding patterns strikingly contrast with the rarity of local plants with a p : c < 1. The *S. cancellata* nymphs performed best on artificial diets matching their intake targets, and failed to gain weight on all tested local plants except an invasive grass with a relatively low p : c. If nymphal fat content predicts that of adults, environmental carbohydrate availability probably limits the migratory
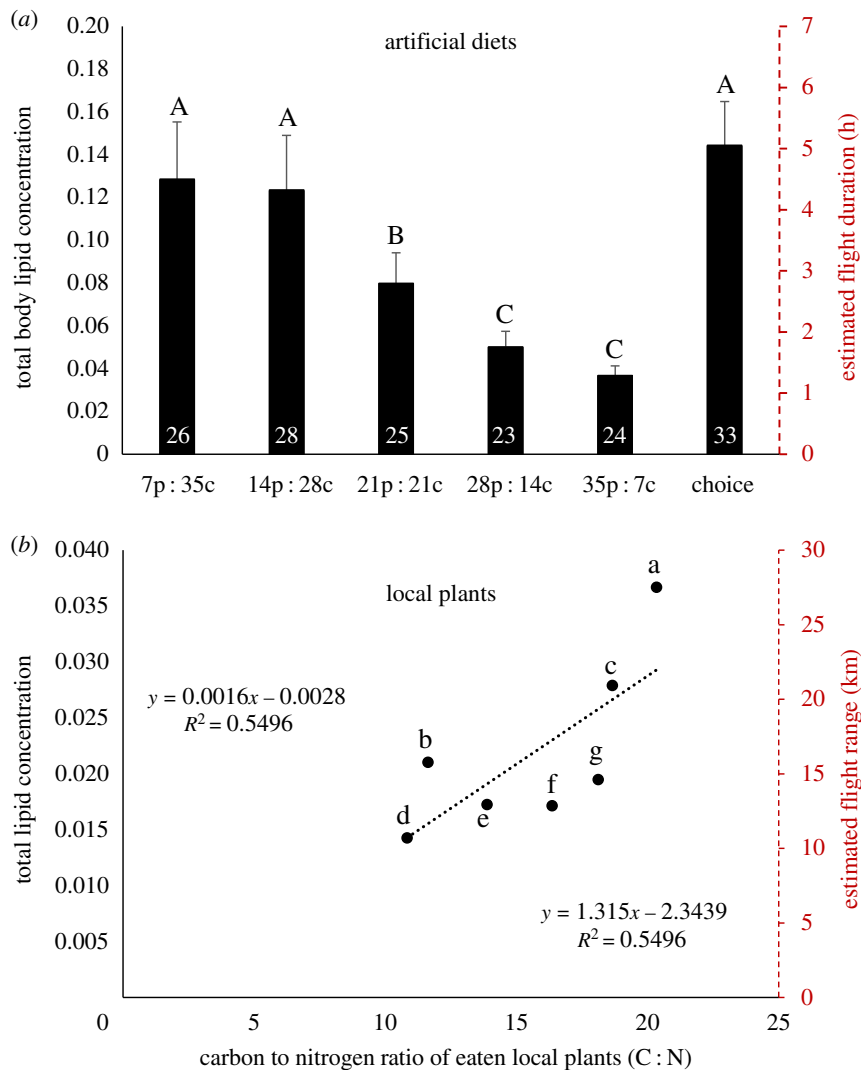
Figure 4. Effects of diet on lipid concentration (mg of lipid weight per mg dry mass) and estimated flight capability for field-collected *S. cancellata*. (*a*) Locusts with a choice of diets (intake target experiments) or feeding on a single artificial diet with a p : c of 1 : 2 or lower, had higher lipid content than locusts confined to feeding on diets with 1 : 1 or higher p : c (ANCOVA using dry mass as a covariate, $F_{5,153} = 36.886$, $p < 0.001$,). (*b*) Lipid content of locusts reared on local plants increased with relative C : N content of the plants.

potential of adult locust swarms. Together, these results demonstrate that macronutrient availability is an important factor affecting physiological performance in the field for this outbreaking migratory species.

(a) Evidence for carbohydrate-limited growth and survival in the field

Our data are consistent with the hypothesis that poor plant carbohydrate availability contributes to limitations on physiological performance and survival of *S. cancellata* in the Gran Chaco region of Paraguay. Multiple experiments demonstrated that outbreaking *S. cancellata* nymphs were 'carbohydrate hungry' and that the plants available to them generally had p : c that were higher than those that are optimal for nymphal growth rate and survival as well as lipid accumulation. First, marching locusts fed primarily on the highest carbohydrate content diets available, mostly ignoring high protein or more balanced options (figure 1*a*). Second, field-collected nymphs selected a ratio of 1 : 2 p : c when given the choice with artificial diets, yet most available plants at the field sites had a p : c of more than 1 (electronic supplementary material, figure S1). Third, field-collected locusts offered a choice among seven

local plants preferred foods with the highest carbohydrate content (figure 3; electronic supplementary material, figure S4).

Similarly, several lines of evidence suggested that a dietary p : c of approximately 1 : 2 improved performance, but that locusts were not able to achieve this balance on field plants. There was evidence that extremely carbohydrate-biased diets are poor food for *S. cancellata*, as locusts on the lowest p : c diet gained less mass (figure 2*b*). However, locusts confined to single artificial diets had increasingly higher survival on diets with lower p : c (figure 2*a*). Out of all the common local plants surveyed, including all those observed to be eaten by locusts, only one had a p : c ratio lower than 1 : 2 intake target (figure 1*b*). When confined to single local plants, locusts only gained mass on the plant with the highest C : N and highest carbohydrate content (figures 2*d* and 3*a*), suggesting that macronutrient content was a major factor determining performance on local plants.

It is sometimes stated in the literature that plants are carbohydrate biased and herbivores are protein limited [31]. Certainly, this is true in some regions, for example, the p : c of plants in a mixed-grass Texas prairie was approximately 0.2 [32]. In this study, we found that the majority of plants in the Gran Chaco are protein-biased (figure 1*b*; electronic

supplementary material, table S1). One explanation for this disparity is that the Chaco forest contains many plants that are nitrogen fixers [33]. Another is that some other studies have measured total carbohydrate, which includes structural carbohydrates such as cellulose and lignin that have low digestibility and contribute little to carbohydrate absorption in locusts and most other insect herbivores [34]. Also, some studies have measured whole plants, while we measured the leaves, which are the primary food of locusts in the wild. Other recent studies of locust nutritional ecology have also found that the p : c intake targets for locusts tended to be lower than the p : c of available plants [18,21,35].

(b) Evidence for carbohydrate-limited lipid accumulation and migratory capacity

Migration of adult locusts to new food sources rely on lipid stores that are accumulated during their nymphal and adult development [36–39]. *Schistocerca cancellata* confined to the carbohydrate-biased artificial diets had the highest lipid contents and these lipid contents were similar to those of locusts allowed to self-select their macronutrient ratio (figure 4*a*). Lipid contents of *S. cancellata* nymphs confined to single local plants increased with plant C : N, but were well below those measured for locusts with access to low p : c artificial diets (figure 4*b*), suggesting that most local plants lack sufficient carbohydrate to support the deposition of high amounts of lipid.

Nymphs with low body lipid contents (collected in the field, or fed high p : c diets) have less fuel for migration [40]. We estimated the migratory flight time and distance (figure 4) of *S. cancellata* collected from the field or reared in the laboratory on artificial or local plant diets (see the electronic supplementary material, methods for details of calculations). Young, recently eclosed adults have slightly lower lipid contents than fourth and fifth instar nymphs in the laboratory, (electronic supplementary material, table S2), so we assumed that young adults of *S. cancellata* in Gran Chaco would have similar lipid concentrations as we measured in fourth and fifth instar nymphs on local plants. Based on our calculations, lipid contents of recently eclosed adults in the field or fed on local plants in the laboratory can only sustain lipid-based flight for approximately 1 h and a distance of approximately 15 km, while locusts able to select from two artificial diets should be able to fly for 5 h and fly over 70 km (figure 4). Our estimated flight durations match well to prior estimates [41], who directly measured flight duration and total lipid content before and after flight. Besides being the key migration fuel, lipids are essential for reproduction, egg maturation and embryo development, and reproductive females must have access to high carbohydrate nutrition in order to produce and lay viable eggs. Locust embryo development is highly reliant on lipid energy stores [42,43]. Thus, the need for carbohydrates to synthesize lipids to support female reproduction may be an additional reason that late-instar *S. cancellata* prefer diets with relatively high carbohydrate content. However, while these calculations should be correct for recently eclosed adults, it is important to note that most lipid in mature adult locusts studied in the laboratory is derived from food eaten in the adult stage [39], and it is plausible that young adults are able to fly to habitats that enable them to find sufficient carbohydrate to accumulate sufficient lipid for longer migration and higher egg production.

(c) Evidence that local Gran Chaco plants are challenging food for *Schistocerca cancellata*

The performance of *S. cancellata* nymphs was better on artificial diets than local plants. More than 80% of locusts survived on artificial diets, and less than 50% of locusts survived on the plants. Average mass gained during the 6 day experiment for locusts consuming artificial diets was approximately 30% whereas nymphs that consumed local plants decreased their mass by approximately 8%. Locusts feeding on artificial diets had up to 5× higher lipid contents than locusts feeding on local plants. The low performance of *S. cancellata* when feeding on local plants compared to artificial diets may be owing to multiple contributing factors including structural and chemical defences of plants, in addition to imbalanced nutrients. Most of the Gran Chaco plants are perennials that are generally well-defended against herbivory. *Grabowskia* sp. are in the Solanaceae, which are well known to be chemically defended. Celestraceae can be defended by dulcitol, terpenoids and isoprene [44]. *Prosopis* sp. are known to be defended by piperidine alkaloids and shikimic acid metabolites [45]. *Celtis* sp. are thought to be defended by leaf toughness (reviewed in [46]), and *Digitaria* sp. by accumulation of silica in the leaves [47].

(d) Environmental change and locust outbreaks

Several components of anthropogenic change may create favourable locust habitats, including elevated CO_2, habitat fragmentation and invasive vegetation. Elevated atmospheric CO_2 tends to increase plant carbohydrate content [48], potentially enhancing the performance and outbreak potential of locusts. Much of the Gran Chaco forest is being converted to agriculture, especially for cattle pastures [49–52]; because locusts performed best on an invasive grass, our findings suggest that outbreaks in this expansion zone may also be facilitated by deforestation. Deforestation in the arid Chaco promotes growth of poorly defended grasses [51], and causes a loss of carbon from the soil [53], potentially lowering plant p : c [52] and improving locust performance.

These patterns for *S. cancellata* parallel observations for locusts in other parts of the world where there is substantial evidence for anthropogenic influences promoting outbreaks. In inner Mongolia, erosion-triggered loss of soil N promotes outbreaks of *O. asiaticus*, partly by increasing the availability of plants with relatively low p : c [16,17,54]. Enhanced deforestation and agriculture in the beginning of the 2000s increased the grassy habitats of the Yucatan in Mexico, elevating populations of *Schistocerca piceifrons* ([9] and the citations within). Similarly, deforestation in Madagascar has been shown to promote outbreaks of red locusts (*Nomadacris septemfasciata*, [55]), potentially through creation of migratory pathways and/or availability of locust-favourable vegetation. Lastly, Deveson [56], linked Australian plague locust outbreaks with increased Australian agricultural development, probably owing to deforestation and expansion of grazing pastures. Together, these findings suggest that land management strategies focused on reducing the availability of poorly defended low- p : c plants may represent an important tool for combatting locust outbreaks, with potential for improving the sustainability of food production and reducing reliance on pesticides.

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