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RESEARCH ARTICLE

Protein-induced mass increase of the gastrointestinal tract of locusts improves net nutrient uptake *via* larger meals rather than more efficient nutrient absorption

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SUMMARY

Increasing the tissue biomass and/or volume of the gastrointestinal tract (GIT) is commonly seen when animals feed on poor-quality diets. This increase can simply permit larger meal sizes, but may also rebalance nutritionally imbalanced ingesta by allowing selective absorption of limiting nutrients. In an insect herbivore, the migratory locust, a synthetic diet with a high ratio of protein to carbohydrate was found to induce mass enhancement of the GIT. When normalised for sex and overall body size, increases to the mass of the foregut and midgut caeca resulted in higher absorption (20–30%) of both protein and carbohydrate when subsequently feeding on three chemically and structurally different grasses. Greater net absorption of macronutrients occurred because these locusts ate larger meals that transited at the same time and with the same digestive efficiency as locusts in which the GIT was not enlarged. Thus, plasticity of the GIT did not improve nutritional homeostasis, but increased the rate of nutrient uptake.

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Key words: phenotypic plasticity, nutrition, digestion, geometric framework.

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INTRODUCTION

It is becoming increasingly appreciated that the gastrointestinal tract (GIT) is a remarkably dynamic organ, exhibiting plasticity in size, structure and function (reviewed by Karasov and Hume, 1997; Starck, 2005; Naya et al., 2007; Karasov et al., 2011). Such plasticity is thought to accommodate fluctuations in the quality and quantity of food over time by sustaining rates of nutrient extraction at a homeostatic norm, supporting both general metabolism and the maintenance costs of the intestine itself (Cant et al., 1996; Hume, 2005). Digestive function and subsequent nutritional outcomes have been modelled using chemical reactor-based theory (Penry and Jumars, 1986; Penry and Jumars, 1987) and considered in terms of optimal digestive strategies (Sibly, 1981; Hume, 1989; Karasov, 1999). However, data are almost always inconsistent with predictions from such models (e.g. Diamond and Hammond, 1992; Yang and Joern, 1994a; Jumars and Martínez del Rio, 1999; Karasov, 1999; Levey and Martínez del Rio, 1999), suggesting problems with the underlying assumptions (reviewed by Karasov, 1999; McWhorter, 2005). Typically, these models have been tested using calorific estimates or by single nutrient analyses, and it is now recognised that animals across all trophic levels balance the intake of multiple nutrients (e.g. Mayntz et al., 2005; Raubenheimer and Jones, 2006; Behmer, 2009; Raubenheimer et al., 2009; Jensen et al., 2012) rather than maximize intake or provide the tissues with a constant flux of energy (Sibly, 1981; Slansky and Wheeler, 1989; Karasov and Hume, 1997; Woods and Kingsolver, 1999). Consistent with this idea, it was recently shown that when locusts were confined to a diet containing an unbalanced ratio of protein to carbohydrate, they exhibited lower digestive enzyme activity for the macronutrient present in relative excess in the diet (Clissold et al., 2010).

An enlarged GIT has generally been associated with animals ingesting poor-quality diets (reviewed by Yang and Joern, 1994b; Karasov and Hume, 1997; Jumars, 2000; Starck, 2005; Naya et al., 2007). By enlarging the GIT when feeding on foods in which nutrients are diluted within an indigestible matrix, it is thought that nutrient supply to the tissues can be maintained by either increasing the efficiency with which each meal is digested or increasing intake rates, albeit with poorer digestibility (e.g. Gross et al., 1985; Hume, 1989; Hammond and Wunder, 1991; Yang and Joern, 1994b; Yang and Joern, 1994a; Karasov and Hume, 1997; Starck, 2005). However, recent research on both locusts and mice has shown the GIT also increases in size when feeding on foods that are energy dense but where the balance of macronutrients is not supplied in the required ratio (Raubenheimer and Bassil, 2007; Sørensen et al., 2010). Interpreting the nutritional outcome in this circumstance is problematic (Raubenheimer and Bassil, 2007; Sørensen et al., 2010). Does upregulation of the physical dimensions of the GIT represent a compensatory response, whereby nutrient homeostasis is maintained by facilitating an increase in the uptake of the deficient macronutrient, or is the response ‘counter-compensatory’, and may actually reduce fitness by maximizing the uptake of all nutrients and supplying the tissues with even more of a macronutrient surplus (Raubenheimer et al., 2005; Boersma and Elser, 2006)?

Explaining how adjustments to the physical dimensions of the GIT affect nutritional outcomes from existing studies is challenging given the numerous co-varying factors typically occurring simultaneously with structural remodelling of the GIT. In the present

study, the relationship between diet, the structural plasticity of the GIT and overall nutrient extraction was investigated in locusts. Experiments were established to determine whether: (1) the increase in GIT mass was uniform or confined to specific regions; (2) enlarging the GIT increased overall macronutrient uptake (ingested minus voided); and (3) the uptake of all macronutrients increased equally, or was confined to a limiting macronutrient only. The overall protocol was (1) to manipulate the plasticity of the GIT using synthetic diets in the fourth and fifth nymphal stadia, and (2) to follow this with a short period of feeding on grasses, during which GIT masses and nutrient extractions were determined. We used grasses to assess the functional implications of changes in GIT mass because *Locusta migratoria* absorbs close to all the available protein and carbohydrate from synthetic diets (Miller et al., 2009). This is most likely due to the lack of structure, in the sense of packaging of nutrients with synthetic diets, rather than the chemical differences with the type of proteins or carbohydrates between natural and synthetic foods. Hence, grasshoppers ingesting dried and ground grasses, where the effects of biomechanical properties have been removed, behave nutritionally identically to grasshoppers feeding on synthetic diets with the same percentage of protein and carbohydrate (Clissold et al., 2006).

MATERIALS AND METHODS

Locusts and diets

Locusta migratoria (L.) came from a long-term culture at The University of Sydney (originally collected from the Central Highlands of Queensland, Australia). Stock locusts were reared in large plastic bins (56×76×60 cm) with 500–1000 locusts per bin in a room kept at 30°C under a 14 h:10 h light:dark photoperiod, with each bin having an additional heat source (250 W heat lamp mounted on the mesh roof of the bin) during the 'lights on' phase. Locusts were provided with *ad libitum* seedling wheat and wheat germ.

Four dry, granular synthetic diets differing in the percentage of protein (P) and carbohydrate (C) (35P:7C, 28P:14C, 14P:28C and 7P:35C) were made as described in Simpson and Abisgold (Simpson and Abisgold, 1985), with protein being a 3:1:1 mixture of casein, bacteriological peptone and egg albumen, and carbohydrate a 1:1 mixture of sucrose and dextrin. All diets contained 4% micronutrients (salts, vitamins and sterols) and 54% indigestible α-cellulose (C8002, Sigma-Aldrich, St Louis, MO, USA) and were ground to a fine powder. Blades of two grass species, *Cynodon dactylon* (L.) Pers. (Poales: Poaceae) and *Themeda australis* (R. Br.) Stapf (Poales: Poaceae), were harvested locally as previously described (Clissold et al., 2010). Seedling wheat, *Triticum aestivum* L. em Thell. (Poales: Poaceae), was grown from seed in a glasshouse for ~20 days. Grass blades were detached at the ligule and the bases were placed in a florist vial with water (Clissold et al., 2004).

Experimental design

We first provide an overview of the experimental design and then describe detailed methodologies for each. In Experiment 1, we determined the relationship between diet and growth across the final nymphal stadia, the mass of each region of the GIT, and subsequent nutrient acquisition from *T. australis*. From the start of the penultimate nymphal stadium to midway through the final nymphal stadium, locusts were provided *ad libitum* access to one of the five treatments and then fed *T. australis* for 24 h. The treatments consisted of four synthetic diets varying in the ratio of protein to carbohydrate (P:C) as described above, and a fifth, where the locusts were allowed to self-select their protein and carbohydrate intake from either 35P:7C *versus* 7P:35C or 28P:14C *versus* 14P:28C. Previous work indicated that an optimal P:C ratio for *L. migratoria* is close to 21:21 (Miller et al., 2009).

In Experiment 2, we separated the effect of treatment diet, GIT mass and subsequent nutrient absorption by generating differences in the GIT without concurrent changes occurring in food intake, development rate or the size or composition of the remainder of the body, and then determined the response to one of three grasses that differed in their protein and carbohydrate composition (P:C): *Cynodon dactylon* [which has a P:C ratio that is closest to optimal for *L. migratoria* (*P*=C)], *Triticum aestivum* (*P*>C) or *T. australis* (*P*<C). At the end of the experiment, the dry mass of each region of the GIT was determined and correlated with feeding behaviours and the rate and efficiency of protein and carbohydrate absorption from each grass. Absorption was defined as the difference in protein or carbohydrate measured in the ingesta and faeces.

Experiment 1: effects of diet on allocation to the GIT and response to *T. australis*

Equal numbers of each sex of newly moulted (within 4 h of ecdysis) fourth-instar *L. migratoria* nymphs whose mass was within a standard deviation of a previously weighed population were randomly allocated to each treatment diet. Locusts were placed alone in clear plastic boxes (17×12×6 cm, length × width × height) containing water, a metal perch and the experimental diet (~10–11 per diet treatment, total *N*=53). All experiments were carried out at 31.5–32.5°C under a 14 h:10 h light dark photoperiod. Nymphs were provided with water and a known amount of fresh synthetic diet (~200 mg) daily until Day 3 of the fifth stadium (Day 0 is day of moulting), when the synthetic treatment diet was exchanged for *T. australis*. A known mass of grass (~1.6 g) was provided and the locusts were allowed to feed for 24 h. At the end of the 24 h, all remaining grass was removed and nymphs were allowed to feed on the initial synthetic treatment diet, and were provided with water until the meals of grass had passed through the GIT (~5 h). At this point, nymphs were killed, and the gut was removed by severing the abdomen between the last two segments, pulling the head until the cervical membrane ruptured and then gently removing the entire gut. The foregut (F), midgut caeca (Ca), midgut ventriculus (M) and hindgut (H) were separated.

Once detached, each GIT section was slit open and washed in insect saline (125 mmol l^{-1} NaCl, 4 mmol l^{-1} KCl, 5 mmol l^{-1} $CaCl_2$, 2 mmol l^{-1} KH_2PO_4, 20 mmol l^{-1} Hepes, pH 7.5) to remove any contents before being blotted dry. Following lyophilization, the mass of each section was weighed to an accuracy of 0.1 μg (Mettler, Melbourne, Australia). All faeces were removed from the container, and the faeces derived from grass were separated. Three response variables were determined for the period (24 h) during which the locusts were confined to the grass diet: (1) intake (measured directly as described below), (2) absorption of nutrients from the GIT (intake minus faeces) and (3) efficiency of absorption (with intake minus faeces as the dependent variable and intake as the covariate), following the logic of Raubenheimer and Simpson (Raubenheimer and Simpson, 1992). Intake of total dry matter was derived by subtracting the dry mass of the remaining uneaten grass from an estimate of the initial dry mass provided. The latter was calculated by fresh-weighing the grass and using a regression based on aliquots of fresh grass that were weighed, lyophilized and reweighed. These aliquots came from subsets of blades of grass that were collected daily. Absorption of protein and carbohydrate was determined from the percentage of protein or carbohydrate in the ingesta less that remaining in the faeces (see below).

Experiment 2: effect of GIT size on intake and feeding behaviour on different grasses

Locusts were randomly allocated within an experimental design balanced by treatment diet (28P:14C or 14P:28C), sex and grass species (*C. dactylon*, *T. aestivum* and *T. australis*) (~11–12 per treatment, N=132) and treated as described above. Meal duration and intermeal interval were determined by manual inspection for all locusts when feeding on the grasses. Feeding behaviour was recorded at 1 min intervals for 3 h, commencing at least 4 h after the nymphs had been provided with grass blades. A meal was considered complete if the locust fed for a minimum of 2 min and did not feed again within 4 min (Simpson, 1982). Intermeal duration is highly correlated with the mean time food is retained within the GIT.

Determination of diet composition and nutrient absorption from the grasses

Total protein, non-structural carbohydrates and cell wall material (neutral detergent fibre) were determined from finely ground (Retsch Mixer Mill MM 400, Haan, Germany) lyophilized samples of the grasses and faeces. Protein was extracted from replicate 10 mg samples of plant and faecal material with 0.1 mol l^{-1} NaOH and determined using the Bio-Rad micro assay (Bio-Rad, Hercules, CA, USA) based on the Bradford assay. Total non-structural carbohydrate was determined colourimetrically from 10 mg plant and faecal samples following extraction with 0.1 mol l^{-1} H_2SO_4 (Smith et al., 1964), using the phenol-sulphuric assay (DuBois et al., 1956). Neutral detergent fibre was determined gravimetrically from replicate 50 mg plant samples using the Van Soest method, omitting sodium sulphite (Van Soest et al., 1991; Clissold et al., 2004). The amount of protein and carbohydrate digested and absorbed was calculated from the amount of each nutrient ingested minus that remaining in the faeces. Fibre is not digested and absorbed by *L. migratoria* (Hochuli et al., 1993) and thus only the amount ingested was calculated. For the grasses used in Experiment 2, leaf mass area was determined as a surrogate measure of toughness (Read and Stokes, 2006; Onoda et al., 2011). Ten leaves from each grass were scanned (Kyocera 4500i, Sydney, Australia) and then the leaves were lyophilized to a constant mass and weighed.

Statistical analysis

For each dietary treatment, the mass of each section of the gastrointestinal tract was compared using ANCOVA, with body mass minus the mass of the entire GIT as the covariate. Although we were interested in absolute differences in size, we used ANCOVA as this removed the effect of sex from all analyses. All differences due to sex were explained entirely by body mass and there were no interactions between sex and any of the other factors. ANCOVA was used to test whether the total mass of the GIT varied between treatments while adjusting the covariates of body mass minus GIT. Responses (intake and absorption of protein and carbohydrate) of the synthetic diets and the grasses of insects pre-treated as described for both experiments were compared using ANCOVA (Raubenheimer, 1995), with body mass at the end of the experiment as the covariate. The efficiency of absorption was analysed using ANCOVA with faeces as the main effect and intake as the covariate (Raubenheimer, 1995).

In Experiment 2, to ensure the effects observed were due to the size of the GIT rather than any effects of the dietary treatment *per se*, the relationship within each dietary treatment was compared by removing the effect of body size on GIT mass, intake and macronutrient absorption. The effect of body size was removed by comparing the residuals of the combined masses of the foregut and caeca ($M_{R,FCa}$) with the residuals for nutrient intake and absorption generated by ANCOVA, with dietary treatment and grass as factors. The masses of the foregut and caeca were highly correlated and these were combined rather than used separately. Feeding behaviours (mean meal and intermeal durations) were compared using ANCOVA, with body size as the covariate. Again, we investigated the trends within a dietary treatment by examining the relationship between the relative size of the foregut and/or caeca and feeding behaviour. All analyses were undertaken using SYSTAT 12 (Systat Software, Chicago, IL, USA), with ANOVA and ANCOVA performed following the techniques outlined in Quinn and Keough (Quinn and Keough, 2002). Prior to all analyses, box plots were used to check for normality and homogeneity of variances across the treatments. For ANCOVA, the interaction term between the covariate and the dietary treatment factor was included in the initial model to test the equality of slopes.

RESULTS

Experiment 1

Masses of regions of the gastrointestinal tract

The entire GIT was heavier in locusts consuming diets where protein was supplied in excess relative to carbohydrate (P:C 35:7 and 28:14) than for the other treatments ($F_{4,52}$=5.29, P<0.001). This difference was due to increases in the foregut and caeca (Fig. 1; supplementary material Table S1). Compared with locusts allowed to self-select their protein and carbohydrate intakes, the foregut and caeca of nymphs fed 35P:7C were 80 and 30% heavier, respectively, and 40 and 20% heavier for nymphs confined to 28P:14C. Across treatments, mass of the GIT (M_{GIT}) was highly correlated with protein intake (both corrected for body size) and to a lesser degree with total food intake, but showed no relationship to the amount of carbohydrate eaten [total food ingested (I_F), M_{GIT}=0.02I_F+7.49, $F_{1,55}$=26.86, P<0.001, r^2=0.32; P intake (I_P), M_{GIT}=0.06I_P+9.10, $F_{1,55}$=188.46, P<0.001, r^2=0.77; C intake (I_C), $F_{1,55}$=0.16, P=0.693, r^2=0.003].

Locusts of both sexes varied in mass with dietary treatment (treatment: $F_{4,47}$=11.05, P<0.001; sex: $F_{1,47}$=16.20, P<0.001; treatment × sex: $F_{4,47}$=1.49, P=0.220; Fig. 2, inset), with those treated on 7P:35C being lighter (P<0.02) than those on all diets except 35P:7C. All nymphs consumed similar amounts of food ($F_{4,52}$=2.31, P=0.071), but as the foods differed in the ratio of P:C, nymphs confined to the 35P:7C diet ingested the most protein and the least carbohydrate (Fig. 2). Similar amounts of carbohydrate were ingested by nymphs on both of the high carbohydrate diets and those allowed to self-select (P>0.5). Nymphs regulating their intake of protein and carbohydrate composed their intake so that a mean (±s.e.m.) of 1.09±0.06 g carbohydrate was consumed for every gram of protein (i.e. 1P:1.1C) regardless of diet pairing (ANOVA: P ingested, P=0.185; C ingested, P=0.308).

Locust performance: rates of nutrient intake, absorption and efficiency of absorption

When feeding on *T. australis*, locusts that had been feeding on either of the diets where protein was oversupplied relative to carbohydrate consumed significantly more (~30–40%) than locusts treated on either of the diets where carbohydrate was supplied in excess of protein ($F_{4,47}$=6.92, P<0.001; Fig. 3A). Locusts that had been allowed to self-select their intake of protein and carbohydrate consumed a similar amount of *T. australis* as locusts on all other treatments (Fig. 3A). Locusts treated with 35P:7C absorbed a lower ratio of P:C from *T. australis* than locusts treated with the two carbohydrate-biased diets because they digested carbohydrate more efficiently (40.8±0.04%) than locusts on all other diet treatments

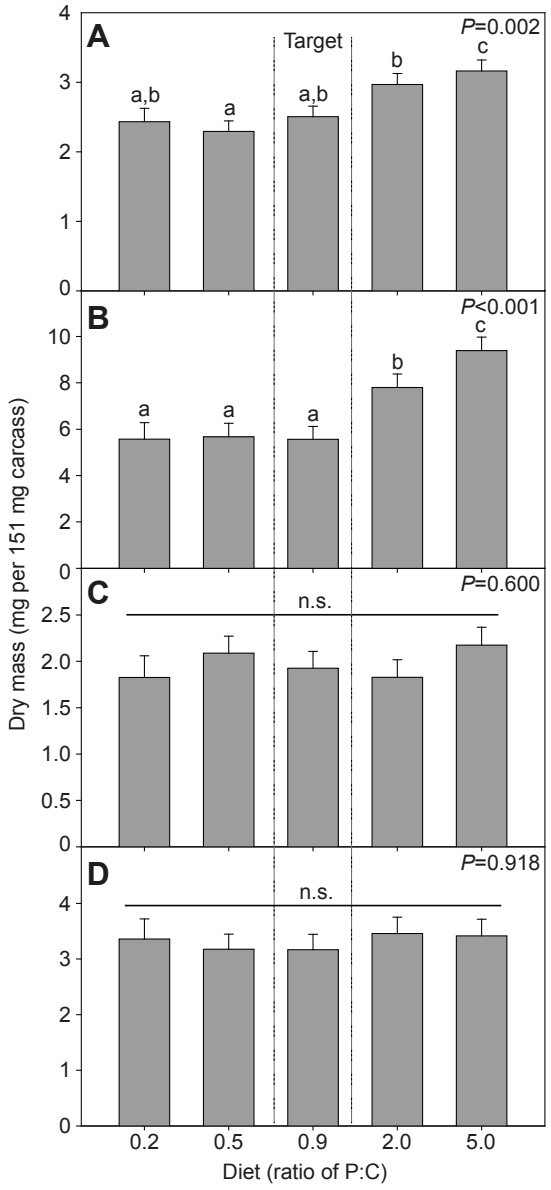

Fig. 1. Dry mass allocation to the different regions of the gastrointestinal tract – (A) foregut, (B) caeca, (C) midgut and (D) hindgut – by locusts after feeding on diets on which they were able to self-select protein or carbohydrate ('target', 1P:1.1C) or where they were confined to a single food varying in the ratio of P:C. Values are ANCOVA adjusted means ± s.e.m. for a 151 mg carcass. *N*=10–12 for each diet treatment. The *P*-values are those for dietary treatment and bars with different letters have significantly different (*P*<0.05) means (Tukey's HSD test); n.s., *P*>0.05.

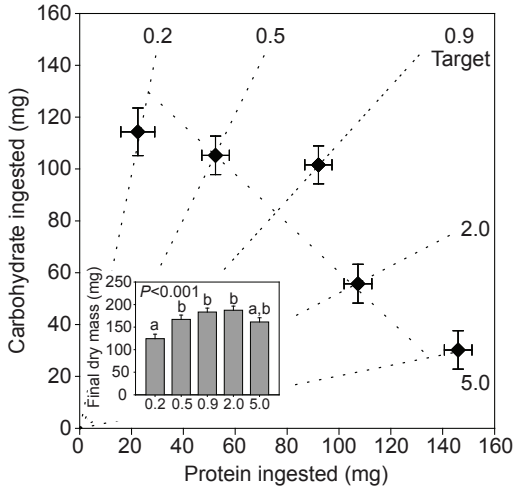

Fig. 2. Protein and carbohydrate intake over the fifth stadium when the locusts were confined to single diets varying in the ratio of P:C or allowed to self-select the ratio of protein and carbohydrate ingested ('target'). The inset shows the final dry mass of locusts on each diet treatment. The *P*-value is for the differences in final body mass for the five dietary treatments, and bars with different letters have significantly different (*P*<0.05) means (Tukey's HSD test).

(27.9±0.03%; Fig. 3B, supplementary material Table S2). Protein was digested with equal efficiency (68.8±0.01%) by all locusts regardless of treatment (supplementary material Table S2).

Experiment 2

Locust performance: rates of nutrient intake, absorption and efficiency of absorption

Nymphs feeding on 28P:14C and 14P:28C were statistically similar in mass (females: 165.1±2.8 mg; males: 135.8±2.7 mg; treatment diet: $F_{1,130}$=0.19, *P*=0.663) and the treatment diets affected the masses of the foregut and midgut caeca but not the remainder of the midgut and hindgut, as found in Experiment 1 (supplementary material Table S1, Fig. S1). In both experiments there was no evidence of the bimodal response reported by Raubenheimer and Bassil (Raubenheimer and Bassil, 2007) for locusts consuming 14P:28C (Levene's test, Experiment 1, *F*=0.15, *P*=0.962; Experiment 2, *F*=0.82, *P*=0.443).

Locusts treated with 28P:14C (with heavier foreguts and midgut caeca) consumed between 20 and 30% more dry matter (or 15–25% when calculated as wet matter) than locusts fed 14P:28C (with smaller foreguts and midgut caeca) (Fig. 4A,B; supplementary material Table S3). The degree to which total nutrient (P+C) absorption increased in locusts fed diet 28P:14C relative to 14P:28C was grass-species dependent ($F_{2,129}$=169.39, *P*<0.001; Fig. 5A). Locusts absorbed most nutrients when feeding from *T. australis* followed by *T. aestivum* and then *C. dactylon* (Fig. 5A). The three grasses differed chemically and physically (Fig. 5B) and although ~90% of the available protein was extracted from all the grasses, the efficiency of carbohydrate extraction differed between grasses (~48% for *C. dactylon*, ~54% for *T. aestivum* and ~62% for *T. australis*). Although the P:C ratio absorbed by locusts from the three grasses differed, there was no interaction between diet treatment and grass species in total nutrient (P+C) absorption (grass × treatment interaction, $F_{1,129}$=0.75, *P*=0.474). Hence, the total amount of nutrients (P+C) differed with diet treatment ($F_{1,129}$=242.10, *P*<0.001), but not their ratio of absorption ($F_{1,129}$=0.07, *P*=0.791; Fig. 5A). The increase in absorption of all nutrients (P+C) across 24 h by locusts with a larger GIT (fed diet 28P:14C) resulted from the ingestion of more food during the 24 h in which the locusts were feeding on the grasses, rather than from the differences in the efficiency with which either protein or carbohydrate was digested (extracted and absorbed) (supplementary material Table S4).

The extent to which diet effects were mediated by differences in the GIT

To establish the extent to which diet-induced effects were attributable to differences in the mass of the GIT, we removed the

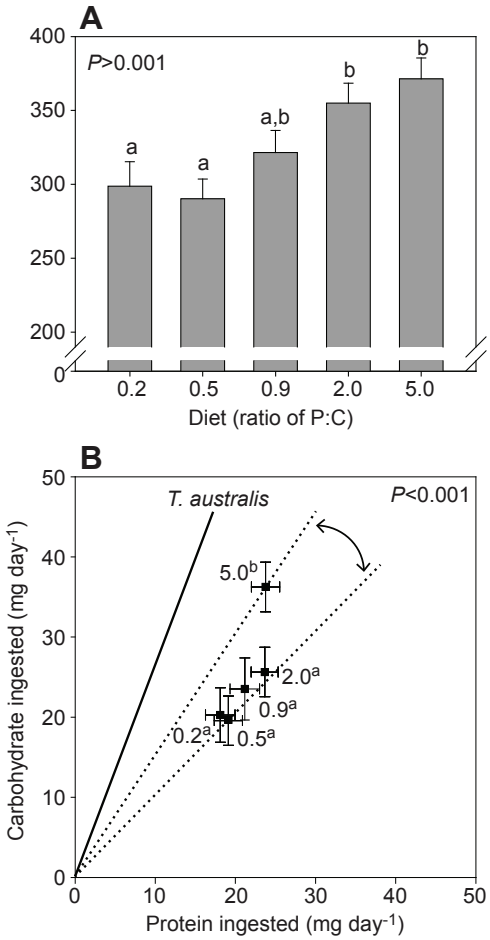

Fig. 3. (A) Total dry matter and (B) protein and carbohydrate extracted (digested and absorbed) in 24 h by locusts feeding on *Themeda australis*, following generation of differences in the size of the foregut and caeca. In B, the solid line gives the ratio of P:C in *T. australis* and the two dotted lines give the two extremes of the ratios of P:C extracted from the grass. Values are ANCOVA adjusted means ± s.e.m. for a 151 mg carcass. *N*=10–12 for each diet treatment. The *P*-values are those for dietary treatment, and bars or symbols with different letters have significantly different (*P*<0.05) means (A) or ratios of P:C absorbed (B) (Tukey's HSD test).

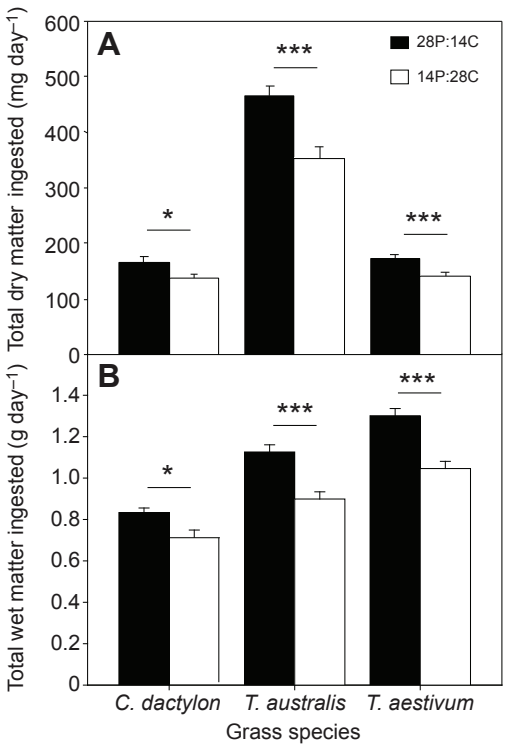

Fig. 4. Total (A) dry and (B) wet matter ingested by locusts in 24 h by locusts feeding on *Cynodon dactylon*, *Themeda australis* or *Triticum aestivum*, following generation of differences in the size of the foregut and caeca. **P*<0.05; ****P*<0.001 (Tukey's HSD test).

effect of body size on both the mass of the foregut plus the midgut caeca and the amount of protein plus carbohdyrate absorbed by taking the residuals of ANCOVA analysis (factors: dietary treatment and grass). A positive relationship was found between the relative mass of the foregut plus midgut caeca ($M_{R,FC}$) and both relative total dry matter intake ($I_{R,DM}$) and relative nutrient absorption ($A_{R,PC}$) ($I_{R,PC}$=5.29$M_{R,FC}$+3.30, $F_{1,129}$=5.80, P=0.021; $A_{R,PC}$=10.24$M_{R,FC}$+0.53; $F_{1,129}$=57.65, P<0.001; Fig. 6), regardless of dietary treatment ($I_{R,PC}$: $F_{1,129}$=0.41, P=0.522; $A_{R,PC}$: $F_{1,129}$=0.01, P=0.915). The residuals of the foregut and midgut caeca were highly correlated (P<0.001), thus these relationships were significant when either section of the GIT was used singly or when combined.

Feeding behaviour

Meal durations were on average 27% longer for 28P:14C-treated nymphs (with larger foreguts and midgut caeca) than for 14P:28C-treated nymphs ($F_{1,129}$=6.19, P=0.014), but intermeal durations did not differ with dietary treatment ($F_{1,129}$=0.45, P=0.502; supplementary material Table S5). Both meal duration and time between meals were grass-species specific (Fig. 7; supplementary material Table S4). Locusts spent longer eating a meal of *T. australis* than the other grasses, and the longest intermeal intervals were found following meals of *T. aestivum* (Fig. 7; supplementary material Table S5). A positive relationship was found between the mass of the foregut (M_F) (where the bulk of the meal is stored upon ingestion) and meal duration (T_{meal}), and between the mass of the foregut plus midgut caeca (M_{FC}) and total food intake (I_{food}) (T_{meal}=0.87M_F+4.07, $F_{1,129}$=8.06, P=0.005; I_{food}=0.006M_{FC}+3.98, $F_{1,129}$=21.75, P<0.001). No relationship was found between intermeal interval and the mass of the foregut plus midgut caeca ($F_{1,129}$=0.43, P=0.512). We modelled the potential uptake rates of protein and carbohydrate using the duration of time spent feeding as a surrogate for total dry matter intake (Fig. 7). We then corrected this for the amount of protein plus carbohydrate actually absorbed (% digested) (Fig. 7, inset). The model indicates that: (1) for each grass, increased nutrient absorption for locusts with heavier GIT occurred because of differences in intake rather than the slight differences in the frequency with which meals were ingested, and (2) absorption rates were highest on *T. australis* as nymphs took the largest meals on average at the same frequency as locusts ingesting *T. aestivum*, and lowest in *C. dactylon*, on which average meals were similar in size to those taken from *T. aestivum* but eaten less frequently (Fig. 7).

Grass chemical and biomechanical properties

In Experiment 1, *T. australis* contained 9.3±0.5% protein and 27.4±1.1% carbohydrate by dry mass (Fig. 3B). *Themeda australis* differed chemically between the two experiments, reflecting changes

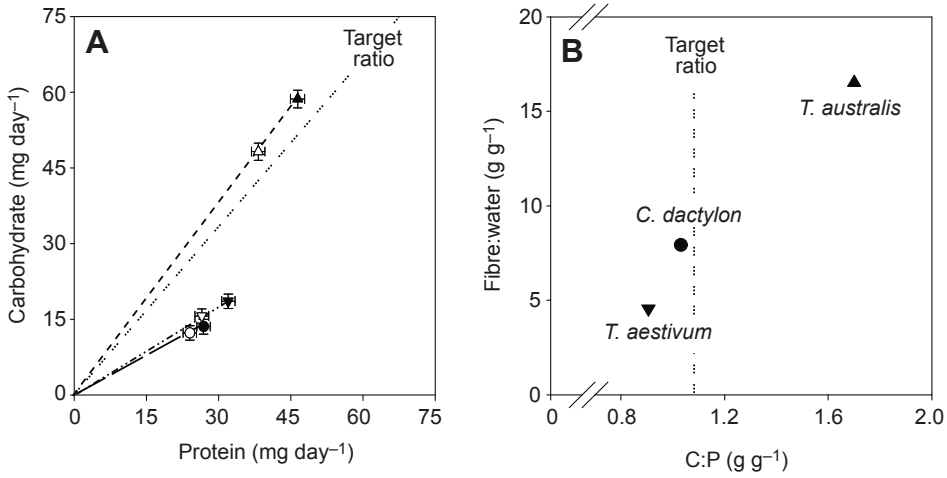

Fig. 5. (A) The ratio and amount of protein and carbohydrate extracted (digested and absorbed) in 24 h by locusts feeding on *Cynodon dactylon* (circles), *Themeda australis* (triangles) or *Triticum aestivum* (downward-pointing triangles), following generation of differences in the size of the foregut and caeca. Filled symbols, 28P:14C; open symbols, 14P:28C. *N*=45 for each grass and *N*=68 for each diet treatment. (B) Physiochemical properties of the three grasses. For all parameters determined, the grasses were significantly different (P<0.05).

with leaf ontogeny given the time between the two experiments. In Experiment 2, the ratio of P:C was significantly lower ($F_{1,19}$=18.22, P=0.001) as the concentration of protein was greater; in addition, the amount of water per unit dry matter was ~25% higher ($F_{1,19}$=13.13, P=0.002). In Experiment 2, all three grasses differed physically and chemically (Table 1, Fig. 5B). The average leaf mass area of *T. australis* was almost double that of *C. dactylon* and three times that of *T. aestivum* ($F_{2,27}$=174.25, P<0.001), and *T. aestivum* had almost double the water per unit dry matter of *C. dactylon*, with *T. australis* having the least ($F_{2,34}$=637.95, P<0.001). Within the dry matter, the concentration of carbohydrate was the same in all grasses (25.9±0.7%; $F_{2,12}$=0.02, P=0.982), but protein ($F_{2,12}$=11.07, P=0.002) and fibre ($F_{2,12}$=5.80, P=0.024) varied. *Themeda australis* contained least protein (16.0±0.6%) and the most fibre (39.9±0.8%), and *T. triticum* had the most protein (27.5±0.5%) and least fibre (36.8±0.8%), with *C. dactylon* having intermediate amounts (protein: 25.7±0.5%; fibre: 38.8±0.5%; Table 1). This resulted in the digestible nutrients, protein and carbohydrate, being most concentrated in *T. aestivum* and most dilute in *T. australis* (Fig. 5B).

DISCUSSION

This study demonstrates that increasing the mass of the GIT serves to maximize the uptake of all nutrients rather than redress nutrient imbalances. Locusts confined to diets in which protein was supplied in a higher than optimal concentration relative to carbohydrate had heavier GITs than locusts either allowed to self select their protein and carbohydrate intakes or confined to diets with a surplus of carbohydrate relative to protein. The GIT was heavier because of increases in the masses of the foregut and midgut caeca (Fig. 1). These changes were associated with a 20–40% increase in absorption of nutrients over 24 h when feeding from three biomechanically and chemically different grasses (Fig. 4). Although the efficiencies with which protein and carbohydrate were absorbed were grass-species specific (Fig. 5A), greater absorption of macronutrients occurred because locusts with heavier GIT ingested larger meals, which were absorbed with the same efficiency and with no change in the time food was retained in the GIT (Fig. 7). Consequently, increases to the mass of the foregut and midgut caeca on a high P:C diet led to the absorption of limiting carbohydrate at the potential cost of supplying the tissues with even more protein (Fig. 5A).

The dry mass of the GIT is often used as a measure of size or capacity (e.g. Yang and Joern, 1994b; Naya et al., 2007; Sørensen et al., 2010). However, interpreting increases in dry mass in terms of adjustments to the capacity of the GIT is problematic because the relationship between dry mass and volume is not straightforward. In vertebrates, increases to both the surface area (e.g. Dykstra and Karasov, 1992) and thickness of the tissues have been found to accompany increased mass of the GIT (reviewed by Starck, 2005). In the present study, increases in mass of the foregut most likely represented a volumetric increase rather than changes to the thickness of the foregut tissue. This follows from the close association seen between the extent of change in foregut mass on a high-protein diet and the similar proportional increases in mass of food consumed and average meal duration. We suggest that the commensurately increased mass of midgut caeca allowed these larger meals to be digested and absorbed at the same rate as on lower-protein diets. Previous work has shown that the anterior arms of the caeca can vary greatly in mass with diet and development, while the mass of the posterior caecal arms and ventriculus remain relatively constant (Chapman, 1988). Increased mass of the anterior caecal arms is likely to enhance both the activities of digestive enzymes and the rates of nutrient absorption.

The precise nature of GIT remodelling is thought to determine the relationship between the extraction of nutrients and the rate at

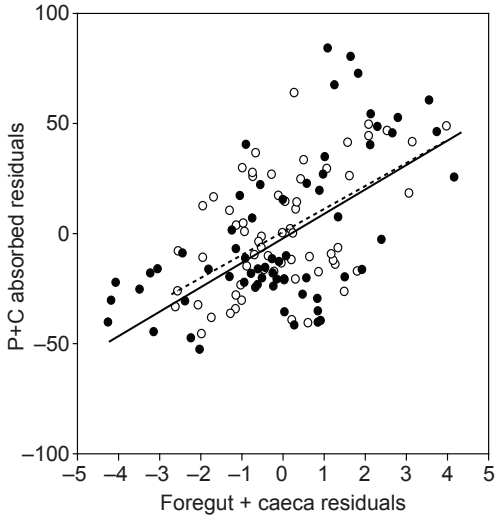

Fig. 6. Locusts with larger gastrointestinal tracts than were explained by body size had a corresponding increase in nutrient absorption that was also not explained by body size. This can be seen by plotting the residuals of ANCOVA analysis (with dietary treatment and grass as factors and body size the covariate).

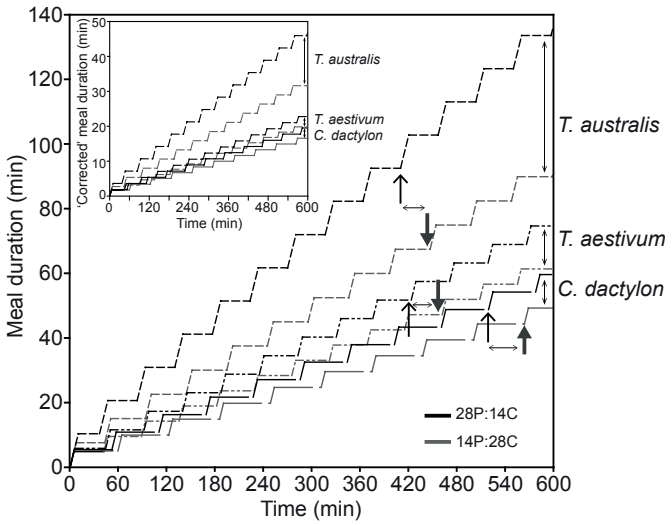

Fig. 7. Using the duration of time spent feeding as a surrogate for intake, we modelled the potential uptake rates and then corrected this for the macronutrient (P+C) content and the percentage digested (inset) to model the rates of macronutrient absorption. Although we did not measure meal size, when the meal duration is adjusted for macronutrient content digested, this is highly correlated with macronutrient absorption over the 24 h the locusts ate the grasses (Fig. 4C), as illustrated by the vertical double-headed arrows. The regular arrows (thick, locusts ingesting 14P:28C; thin, locusts ingesting 28P:14C) indicate the *n*th meal and demonstrate that differences in nutrient absorption for *T. australis* and *T. triticum* can only have been due to differences in meal sizes, as intermeal durations were the same (as indicated by the horizontal double-headed arrows).

which this occurs. The flow of food through the GIT is considered a function of GIT volume, the mobility of the substrate, the initial amount of substrate, the rate of enzymatic hydrolysis (i.e. how quickly can the substrate be digested) and subsequent absorption. Modelling the GIT as a tube shows that volume can be increased by increasing the length and/or diameter. It is thought that lengthening the GIT will increase the efficiency of nutrient absorption, as the surface area to volume ratio is maintained. Consequently, intake rate is maintained, but as each meal takes longer to pass through the GIT, a greater proportion of nutrients can be extracted. However, if the increase is to the diameter, then the time a meal is retained should also increase proportionately or, if retention time remains constant, then the efficiency of absorption should decline (Sibly, 1981; Yang and Joern, 1994a; Raubenheimer and Simpson, 1996; Karasov and Hume, 1997; Raubenheimer and Simpson, 1998). For each grass species, regardless of meal size, a similar proportion of ingested nutrients were absorbed in the same amount of time (Fig. 7). It is difficult to envisage the presumed change in volume occurring because the foregut grew longer, as the size of the locusts from both treatments was the same and the foregut is an unfolded tube. Thus, the most likely explanation is that the foregut increased in diameter, and that digestive efficiency and intermeal durations were similar regardless of meal sizes because greater surface area in the caeca allowed for increases to the production of digestive enzymes, nutrient transporters and/or surface area for passive nutrient absorption. Little is known regarding the regulation of absorption in insects; however, studies on mammals have shown that intestinal nutrient transporters are regulated positively by substrate levels in the diet (Diamond and Karasov, 1987).

Physical plasticity of the GIT has been well described for many taxa (e.g. Hume, 1989; Karasov and Hume, 1997; Starck, 2005; Naya et al., 2007) in response to changes in either energy demand or energy density in the diet, and is thought to be a way of balancing the expensive costs of maintaining the GIT against returns. In mammals it is believed that the GIT accounts for ~25% of digested energy (McBride and Kelly, 1990; Cant et al., 1996), and thus any upregulation is only justified if accompanied by a payoff in calories (Karasov and Diamond, 1983). While this is clearly the case for animals following a period of starvation (e.g. Secor and Diamond, 1998; Hume et al., 2002) or when 'fattening up' for migration (e.g. van Gils et al., 2008), enlargement of the GIT when dietary nutrients are supplied in sub-optimal ratios has been difficult to interpret using energy-based cost–benefit arguments (Raubenheimer and Bassil, 2007; Sørensen et al., 2010).

A previous study by Raubenheimer and Bassil (Raubenheimer and Bassil, 2007) found that locusts ingesting protein-biased diets (28P:14C) had heavier GITs than locusts feeding on a near optimal diet (21P:21C). Locusts ingesting a slightly carbohydrate-biased diet (14P:28C) over the entire fifth stadium showed a bimodal response, with approximately half of the insects having GITs similar in mass to locusts ingesting an optimal diet and half having heavier GITs, similar to locusts on a protein-biased diet. Although some insects partition protein and carbohydrate digestion into different regions of the GIT (Terra and Ferreira, 2012), we found an increase in the masses of the foregut and midgut caeca only for locusts ingesting protein-biased diets (Fig. 1). Similar to the results of Raubenheimer and Bassil (Raubenheimer and Bassil, 2007), in the present study, increased mass of the GIT was associated with increased protein intake, but we did not find a similar increase, nor a bimodal response, in locusts fed a carbohydrate-biased diet (14P:28C).

The functional outcomes of increases in the mass of parts of the GIT when protein-rich/carbohydrate-poor diets are eaten have been difficult to interpret. Does the increase in size facilitate the increased uptake of the deficient nutrient, i.e. a compensatory response, or is the response counter-compensatory, with the GIT growing in response to excess protein intake and, as a consequence, are tissues are supplied with even more of this excess nutrient (Raubenheimer and Bassil, 2007; Sørensen et al., 2008)? Our results show that the increased mass of the foregut and caeca led to an increase in intake

Table 1. Physiochemical properties of the three grasses used in Experiment 2

	Cynodon dactylon	*Themeda australis*	*Triticium aestivum*	*P*
Protein (%)	25.7±0.5^{b}	16.0±0.6^{a}	28.6±0.5^{b}	**0.002**
Soluble carbohydrate (%)	25.9±1.3	25.7±1.6	26.0±1.4	0.982
Water:dry matter (g g^{-1})	4.89±0.08^{a}	2.43±0.09^{b}	7.90±0.16^{c}	**<0.001**
Cell wall (%)	38.8±0.5^{a}	39.9±0.8^{a}	36.8±0.8^{b}	**0.024**
Leaf mass area (mm^{2} g^{-1})	25.1±1.0^{a}	45.4±1.6^{b}	16.5±0.3^{c}	**<0.001**

Data are means ± s.e.m. Different letters indicate means that are significantly different (P<0.05, Tukey's *post hoc* comparison). Bold *P*-values indicate significant differences between grasses.

and nutrient absorption. This is a counter-compensatory effect in terms of maintaining nutritional homeostasis, i.e. the nutritional conditions triggering the increase in mass of the GIT result in the tissues being supplied with even more of the nutrient (protein) in excess of requirements (Fig. 5A, results for *C. dactylon* and *T. aestivum*). It seems counterintuitive that an animal would increase investment in an organ that results in increased uptake of a nutrient surplus to requirements with potential reductions in fitness (Raubenheimer et al., 2005; Boersma and Elser, 2006; Lee et al., 2008). However, it has been argued that insects best ascertain their nutritional status post-absorptively, and thus it is advantageous to rapidly absorb and then subsequently excrete surpluses rather than passing excess nutrients through the digestive tract (Simpson and Raubenheimer, 1993). If this was the case, then the GIT should be bigger when carbohydrate was supplied in excess relative to protein, and this did not occur (Fig. 1). Our results might be taken to imply that the long-term cost of ingesting surplus protein is smaller than the cost of ingesting too little carbohydrate. However, it is not clear from existing data why this is, as *L. migratoria* appears to have a very limited capacity to convert excess protein to carbohydrate *via* the deamination of proteins (Raubenheimer and Simpson, 2003).

Locusts feeding on the 35P:7C foods digested and absorbed a greater proportion of ingested carbohydrate from *T. australis* than locusts from all other treatments (Fig. 3B), a result consistent with our previous findings where protease activity decreased when locusts were ingesting a 35P:7C diet (Clissold et al., 2010). This differential release of digestive enzymes occurred in response to very short-term nutrient imbalances, over a period of time that is too short to induce detectable changes in the mass of the caeca (Clissold et al., 2010). The over-ingestion of either protein or carbohydrate with respect to the other resulted in reduced activity of the digestive enzyme for the excess substrate; i.e. when a 7P:35C diet was eaten, amylase activity declined, and when 35P:7C was ingested, α-chymotrypsin activity was reduced (Clissold et al., 2010). Interestingly, in the present study, differences in digestibility only occurred when protein and carbohydrate were very imbalanced with respect to requirements (35P:7C) (Fig. 3B), and we detected no differences in the efficiency with which protein or carbohydrate were digested and absorbed when the locusts were feeding on slightly protein/carbohydrate-imbalanced foods (Fig. 3B, Fig. 5A). However, the masses of the foregut and caeca increased in response to the ingestion of even slight excesses of protein (i.e. 28P:14C) (Fig. 1). Thus, it appears that modulation of digestive enzymes occurs rapidly in response to extreme nutrient imbalances, while changes in mass appear to occur more slowly and only in response to the over-ingestion of protein relative to carbohydrate.

Knowledge of how the GIT functions is an essential component of understanding how behavioural and physiological mechanisms are coordinated by animals in relation to the ecological niche they occupy (Simpson et al., 2010). We suggest that future studies need to consider any changes to digestive function with regard to the optimal requirements of an organism, together with knowledge of the organism's current nutritional status and the supply of nutrients from ingested food (i.e. the ratio of P to C absorbed rather than that in the ingesta).

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