

## RESEARCH ARTICLE

### Insect herbivores can choose microclimates to achieve nutritional homeostasis

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#### SUMMARY

The interaction between temperature and diet quality can affect the life history of ectotherms. The rate and ratio at which protein and carbohydrate are obtained from food are important aspects of diet quality, and insects have a well-developed capacity to adjust their feeding behaviour and post-ingestive physiology to regulate intake and allocation of these nutrients. If the supply of protein and carbohydrate varies with temperature (e.g. *via* effects on intake, digestion or metabolism), then herbivorous insects can use thermoregulatory behaviour to help achieve nutritional homeostasis. When fed the host grass *Triticum aestivum*, *Locusta migratoria* nymphs absorbed and allocated protein and carbohydrate to growth with the same efficiency at 38°C as at 32°C; however, at the higher temperature, they ingested more food. In contrast, when feeding on *Themeda triandra*, the nymphs absorbed carbohydrate with higher efficiency at 32°C, and protein at 38°C. Using synthetic diets, we induced either a protein or a carbohydrate deficiency in experimental insects and showed that locusts placed in a thermal gradient following a meal of *T. triandra* selected 32°C when deprived of carbohydrate, and 38°C when deprived of protein. This capacity to use thermoregulatory behaviour to redress an imposed nutritional imbalance improved with experience of feeding on *T. triandra*. As predicted, locusts fed *T. aestivum* always chose higher temperatures, irrespective of nutritional state. Our results have consequences for understanding host-plant choice by herbivores and interpreting the effects of changed environmental temperatures and microclimate on animal–plant interactions.

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#### INTRODUCTION

Temperature and nutrition greatly influence life history outcomes for ectotherms through their effect on body size and age at maturity (Angilletta, 2009; Berrigan and Charnov, 1994; Stearns, 1992). These interactions are generally thought to be mediated by the effects of temperature on metabolic rate, which alters energy requirements. For example, growth efficiencies can be reduced with increasing temperatures as energy is diverted away from growth to fuel heightened metabolic processes (e.g. Angilletta, 2009; Kingsolver and Huey, 2008; Miller et al., 2009). However, this classic concept of thermal effects on growth based on energy or caloric budgets may be incomplete because it does not consider the type or balance of nutrients being ingested. Extensive research demonstrates that macronutrient balance, rather than energy *per se*, is a primary determinant of rates of development and growth in animals (Raubenheimer and Simpson, 1993; Raubenheimer et al., 2009; Simpson and Raubenheimer, 2000; Simpson and Raubenheimer, 2012; Simpson et al., 2004). For many herbivores, including locusts, the uptake of the macronutrients protein and carbohydrate influences growth and body mass the most (e.g. Behmer, 2009; Simpson and Raubenheimer, 2012; Simpson et al., 2004).

For many chewing herbivorous insects, final body mass does not vary with temperature in a consistent way, and temperature effects are host plant specific (e.g. Diamond and Kingsolver, 2010; Mousseau, 1997; Walters and Hassall, 2006). For example, Diamond

and Kingsolver (Diamond and Kingsolver, 2010) found that the tobacco hornworm, *Manduca sexta*, grew larger at colder temperatures on their preferred host plant, tobacco, but the slope was reversed for the thermal reaction norm when eating devil's claw, a less preferred host plant. Emerging research has demonstrated that, when herbivores ingest plants, complex interactions between the plants and the herbivores come into play, and protein and carbohydrate are not acquired in the ratio they are ingested (reviewed by Clissold, 2007). This discrepancy is due to not yet fully understood interactions between the anatomy and cellular structure of the leaf, the morphology of the insect's mouthparts and the time food is retained within the gastrointestinal tract (Clissold et al., 2006; Clissold et al., 2009; Clissold et al., 2010). Thus, the plant-specific effects on body mass may occur because temperature affects the rate of feeding and chewing, food transit times and post-ingestive metabolic processing of food (Angilletta, 2009; Du et al., 2007; Huey and Slatkin, 1976) – all factors that have plant-specific effects on the balance of protein to carbohydrate absorbed.

Experiments using synthetic diets have revealed that herbivorous insects have a well-developed capacity to adjust their feeding behaviour and post-ingestive physiology in order to regulate intake and allocation of specific nutrients to maintain nutritional homeostasis (reviewed by Harrison et al., 2012; Simpson and Raubenheimer, 2012). If temperature does alter the ratio of protein and carbohydrate absorbed from the ingested plants, then potentially,

insect herbivores could make use of variations in temperature to regulate their nutrition. In an earlier study, the migratory locust, *Locusta migratoria*, used temperature selection to adjust the trade-off between the rate and efficiency of energy gain (Coggan et al., 2011). *Locusta migratoria* favoured increased metabolic efficiency when food was limited and increased growth rate when food was in excess (Coggan et al., 2011; Miller et al., 2009).

To test the hypothesis that insects can use temperature to regulate the ratio of protein to carbohydrate absorbed from plants, we used an established insect–plant system: *L. migratoria* feeding on two host grasses, *Themeda triandra* (kangaroo grass) and seedling *Triticum aestivum* (wheat). These grasses were chosen because *L. migratoria* absorb different amounts of protein (P) and carbohydrate (C) (Clissold et al., 2013) from each grass. Studies using synthetic diets revealed that *L. migratoria* nymphs achieve optimal growth and development when feeding on a slightly carbohydrate-biased diet (1P:1.1C) (Clissold et al., 2013; Miller et al., 2009). When feeding on kangaroo grass, protein and carbohydrate were absorbed in a ratio (1P:1.3C) very close to this optimum. We expect rates of growth and development will be reduced when feeding on seedling wheat, as protein is massively oversupplied relative to carbohydrate requirements (1P:0.5C) (Clissold et al., 2013).

In the present study, we first determined the host-plant-specific responses to temperature. In laboratory experiments, the effects of temperature on life history variables were ascertained and gravimetric measures were used to quantify the rates of protein and carbohydrate absorbed over the final nymphal (fifth) instar for *L. migratoria* feeding on either kangaroo grass or wheat. Results from the first experiment revealed that the relative rates of protein and carbohydrate absorption depended on temperature when feeding on kangaroo grass, whereas the absolute rate of absorption of protein and carbohydrate, but not the ratio, were temperature dependent when feeding on wheat. Thus, the potential existed for locusts feeding on kangaroo grass but not wheat to make use of thermal heterogeneity over fine scales to adjust for nutrient-specific imbalances. We tested two predictions: (1) locusts fed kangaroo grass should respond to an imposed imbalance in protein–carbohydrate status by selecting a higher temperature when deficient in protein, and a lower temperature when deficient in carbohydrate; and (2) locusts fed wheat should select higher temperatures irrespective of their protein–carbohydrate status, as higher rates of nutrient acquisition and growth occur at such temperatures. In further trials, we assessed whether temperature selection could improve with experience.

## MATERIALS AND METHODS

### Locusts and diets

*Locusta migratoria* (Linnaeus 1758) (Orthoptera: Acrididae) came from a long-term culture at The University of Sydney (originally collected from the Central Highlands of Queensland, Australia). Between 500 and 1000 nymphs were reared on seedling wheat and wheat germ in large plastic bins (56×76×60 cm) under a 14 h:10 h light:dark photoperiod in a room kept at 30°C. During the ‘lights on’ phase, each bin had an additional heat source (250 W heat lamp) mounted on the mesh roof of the bin allowing locusts to thermoregulate by moving vertically on perches and the sides of the bins.

Leaf blades were harvested from mature *Themeda triandra* (Poales: Poaceae) and from 2-week-old *Triticum aestivum* (Poales: Poaceae) grown in a glasshouse. Three treatment diets were used, with the percentages of protein and digestible carbohydrate as follows: 21%P:21%C (21P:21C, balanced); 35%P:7%C (35P:7C,

low carbohydrate); and 7%P:35%C (7P:35C, low protein). Treatment diets consisted of dry granular synthetic foods differing in the ratio of protein (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 the remainder consisted of indigestible cellulose (54%), prepared as described previously (Simpson and Abisgold, 1985).

### Insect responses to temperature and diet

#### Experiment 1: determination of nutrient intake, absorption and growth

We measured the protein and carbohydrate (digested and absorbed) from the gut (measured as nutrient in ingesta less nutrient in faeces), and subsequent protein and carbohydrate growth (a measure of utilization) by locusts at two temperatures (see below for details). We used 32°C, where nutrient utilization efficiency is highest, and 38°C, where development rate is fastest (Miller et al., 2009). Freshly moulted (within 3.5 h) fifth instar male locusts, weighing between 360 and 420 mg, were randomly assigned to treatments ( $N=20$  per treatment), with treatments being temperature (32 or 38°C) and grass species (wheat or kangaroo grass). Locusts were killed within 3.5 h of moulting into adulthood and lyophilized to a constant mass. A known amount of fresh grass was provided daily to allow *ad libitum* feeding and all remaining grass and faeces were removed and stored at –80°C prior to lyophilisation. Two experimental rooms were maintained at either 32 or 38°C ( $\pm 0.1^\circ\text{C}$ ) under a 14 h:10 h light:dark photoregime. Each locust was individually housed within a clear plastic container (7×13.5 cm, diameter × height) so that consumption and faecal output could be determined for each nymph for the duration of the stadium. To minimize differences in leaf chemistry and biomechanical properties over the 24 h that the locusts had access to the grasses, blades of the three oldest fully expanded leaves per stem were detached at the ligule and placed in a vial as previously described (Clissold et al., 2004). Each day, a subset of the harvested grass blades were put aside to determine the ratio of fresh mass to dry mass and the amount of protein and non-structural carbohydrate (supplementary material Table S1). During the experiment, water loss and metabolic changes to the grass blades during the 24 h in which the grass blades were available to the locusts were quantified and values used to correct intake if required (supplementary material Table S2) (Bowers et al., 1991).

Total intake of protein and carbohydrate were calculated from daily food consumption based on the percentage of protein and carbohydrate in the grass. Absorption of protein and carbohydrate was determined as the difference between the mass of nutrient ingested and that remaining in the faeces (Fig. 1) (Clissold et al., 2006; Clissold et al., 2009). Using the Bradford assay, we are able to determine exogenous protein and remove the effect of endogenous nitrogenous waste products, such as amino acids and uric acid in the faeces. The majority of carbohydrates absorbed are respired, thus, those in the faeces are mostly of exogenous origin (the peritrophic membrane is not hydrolysed by our extraction technique). Total growth was defined as final dry mass less initial dry mass, with the initial dry mass being calculated from a regression of dry mass against wet mass derived from a subset of 20 freshly moulted fifth instar nymphs. Protein and lipid growth was measured as the increase in carcass protein and lipid, respectively, estimated from regressions for the protein and lipid content of the subset of 20 freshly moulted fifth instar locusts. Digestive efficiency (absorption/intake) was calculated using ANCOVA-corrected means for nutrient absorption expressed

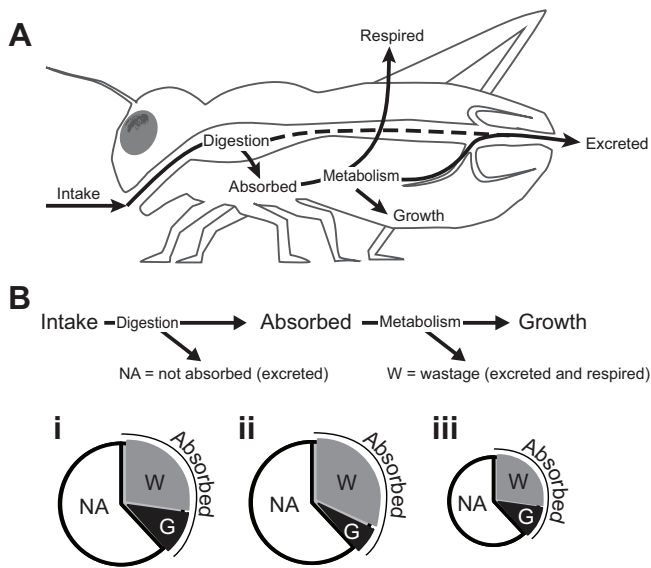


Fig. 1. Schematic diagram outlining the fate of ingested food. (A) Food can either pass through the gastrointestinal tract and be excreted, or be absorbed across the gastrointestinal wall. Of nutrients absorbed, these can be allocated to growth or lost via metabolic processes. (B) Differences in growth result from increased wastage (metabolism) of absorbed nutrients (pie chart i vs ii); or the same proportion of absorbed nutrients are wasted or allocated to growth, but intake or the proportion of nutrients absorbed is reduced (pie chart i vs iii); or intake is reduced and post-absorptive allocation to growth is reduced (pie chart ii vs iii). NA is the fraction of ingesta not absorbed (intake – excreta), W is the fraction of nutrients absorbed but not allocated to growth, and G is the fraction of absorbed nutrients allocated to growth.

relative to nutrient intake, and growth efficiency (growth/initial biomass) was calculated from ANCOVA-corrected means for nutrient specific growth expressed relative to initial nutrient biomass. For example, protein digestibility at 38°C = 100(ANCOVA-corrected mean of protein absorbed at 38°C/mean of protein intake across both temperatures).

Protein and non-structural carbohydrates (available carbohydrate) were determined from lyophilized samples of the grasses and faeces that had been finely ground prior to analysis. Protein was extracted from replicate 10 mg samples with 0.1 mol l<sup>-1</sup> NaOH and determined using the Bio-Rad Protein Microassay for Microtiter Plates based on the Bradford assay (Bio-Rad Laboratories, Hercules, CA, USA). Total non-structural carbohydrate was determined colorimetrically from 15 mg replicate samples following extraction with 0.1 mol l<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> (Smith et al., 1964) using the phenol-sulphuric assay (DuBois et al., 1956). Carcass lipid was determined gravimetrically following three 24 h extractions with 100% chloroform. Previous regression analysis had found that, regardless of diet, the lipid-free dry matter contains the same proportion of protein (0.81) (F.J.C., personal observation) and thus protein was determined by multiplying the lipid-free dry matter by this value.

#### Determining thermal preference

Experiment 2: temperature selection following a meal of either wheat or kangaroo grass by either protein- or carbohydrate-deprived locusts

Freshly moulted fifth instar male locusts were randomly allocated to either the 32 or 38°C constant temperature room (rearing

temperature) and provided with an optimally balanced synthetic diet (21P:21C) (Miller et al., 2009) from Day 0 (day of moult) to Day 3. On Day 3, half the locusts from each temperature were confined to a diet containing 35P:7C and the other half to 7P:35C for 4 h at 35°C, which rendered the locusts either carbohydrate or protein deprived, respectively (see Appendix for methods; supplementary material Fig. S1). Protein- or carbohydrate-deprived locusts were provided with fresh blades of either wheat or kangaroo grass and observed on a thermal gradient plate as described below. This experiment was repeated (trials 1 and 2, ca. 5–10 per treatment, with a total of eight treatments – 2× rearing temperature, 2× nutrient state and 2× grass species – per trial, *N*=122).

#### Experiment 3: can temperature selection performance be improved with experience?

Forty-eight freshly moulted fifth instar male nymphs were provided with kangaroo grass and randomly assigned to either 32 or 38°C ('experienced' locusts) or reared as for Experiment 2 on an optimally balanced synthetic diet (21P:21C) ('naive' locusts). Locusts feeding on kangaroo grass at 32°C were supplied with a 1:1 ratio of protein and carbohydrate, which is higher than optimal, and at 38°C a lower than optimal diet was supplied (1P:1.5C). Regardless of diet treatment locusts were switched between the two temperatures every 3.5 h [the average time interval at which they switch between a diet high in protein to one high in carbohydrate if allowed a choice (Chambers et al., 1995)] from Day 0 (day of moult) to Day 3. On Day 3, locusts were rendered protein or carbohydrate deprived (at 35°C) as previously described and then placed in a thermal gradient with a meal of kangaroo grass. This experiment was repeated (*N*=94).

#### Thermal gradient plates and determination of temperature preference

Thermal gradients were established in a 28°C constant temperature room on six steel plates. Each plate was 700×100×10 mm (length × width × depth), and a thermal gradient was established by the use of 150 and 75 W ceramic heat lamps beneath either end of each plate, producing horizontal temperature gradients ranging from 27 and 45°C, both at the surface and 1 cm above the surface of the plate (Coggan et al., 2011). Temperature profiles of each plate were measured using infrared images (IR Camera S65, FLIR Systems, Wilsonville, OR, USA) and measurements validated with a 0.5 mm beaded wire K-type thermocouple placed on and 1 cm above the plate (YC-747D, Yu Ching Technology, Taiwan). Locusts were confined to a region on the plate between approximately 27 and 45°C using an elliptical wall, 300 mm high and coated in liquid Teflon (Fluon, The Herp Shop, Ardeer, Vic, Australia), as previously described (Coggan et al., 2011). A vial of fresh grass blades was placed on each plate at 35°C and perches of wire mesh were placed at 32 and 38°C, which were equidistant (~12 cm) from the food. The water-filled vial containing the grass blades was an inverted 1.5 ml microfuge tube cut to a height of 15 mm and attached to a 40 mm diameter Petri dish lid. Locust behaviour was captured on digital video (JVC Everio, Yokohama, Japan) and then quantified visually. Locusts alternate between periods of movement and remaining relatively stationary (Coggan et al., 2011), and during the stationary periods, locusts are either feeding or processing ingesta. Thermal preference was recorded once the locust had settled (within 5 min of completing the ingestion of a meal of the grass blades) and remained stationary for a minimum of a further 15 min. To quantify thermal preferences, the plate was divided into five 'zones' of width 3°C; ~27–30.5°C, 30.5–33.5°C, 33.6–36.5°C,



36.6–39.5°C and 39.5–43°C, with locusts being scored as being at 29, 32, 35, 38 or 41°C, respectively. As found previously (Coggan et al., 2011), locusts did not go below 30°C or above 43°C; i.e. they were not constrained in their upper or lower temperature selection by the walls confining them within the thermal gradient. The position of the thorax of the locusts was used as the point of reference. The steel plates were randomly oriented in the room to control for any effects of position.

### Data analysis

Life history outcomes and nutritional behaviours were analysed separately for each grass using ANOVA and ANCOVA, as there were complex interactions between diet and temperature. Nutrient-specific intake, digestion and total growth were compared with ANCOVA using initial biomass as the covariate. In all cases the assumptions for ANCOVA and ANOVA were met. Temperature selection behaviour was compared using ANOVA and  $\chi^2$  tests. Temperature selection from Experiment 2 was initially modelled with trial, rearing temperature, grass species and nutrient state as fixed factors; as trial and rearing temperature were non-significant ( $P>0.5$ , thus, there was a minimal chance of a type II error), these terms were removed from the final model. To investigate the effect of learning on temperature selection for locusts being offered kangaroo grass (Experiment 3), we used ANOVA with knowledge state and nutritional state as fixed factors. Approximately 10% of locusts were excluded from the temperature preference analyses as they failed to ingest a meal of grass within 2 h.

## RESULTS

### Experiment 1: determination of nutrient intake, absorption and growth

Regardless of grass eaten, locusts developed ca. 35% faster at 38°C than at 32°C (5.4 vs 7.6 days, respectively;  $F_{1,76}=450.9$ ,  $P<0.001$ ). However, growth of fifth instar *L. migratoria* nymphs differed with temperature in a host plant-specific way, due to differences in the rate and balance of nutrients absorbed from the gut. For nymphs fed kangaroo grass, although this was ingested 38% faster at 38°C than at 32°C, temperature did not affect the total amount of nutrients (P + C), and hence energy, absorbed ( $F_{1,37}=0.39$ ,  $P=0.580$ ; Fig. 2A, inset). However, the ratio of protein to carbohydrate absorbed differed markedly (Fig. 2A). At 32°C, more carbohydrate than

protein was absorbed than at 38°C (1P:1.5C vs 1P:1.1C, respectively; P:  $F_{1,37}=36.96$ ,  $P<0.001$ ; C:  $F_{1,37}=7.14$ ,  $P=0.011$ ; ratio P:C:  $F_{1,37}=4.77$ ,  $P=0.035$ ; Fig. 2A,B). Once absorbed from the gut, both protein and carbohydrate were converted to biomass with higher efficiency at 32°C than at 38°C (Fig. 2B), and locusts were heavier at 32°C than at 38°C (Fig. 2C).

In contrast, for locusts feeding on wheat, although intake rates were 63% higher at 38°C than at 32°C, there was no difference in the ratio of nutrients extracted at 32 and 38°C ( $F_{1,37}=1.28$ ,  $P=0.266$ ). Approximately 90 and 60% of the ingested protein and carbohydrate, respectively, was absorbed. However, more of both protein and carbohydrate were extracted at the higher temperature (P:  $F_{1,37}=25.6$ ,  $P<0.001$ ; C:  $F_{1,37}=12.8$ ,  $P=0.001$ ), leading to more nutrients in total being absorbed over the fifth stadium at 38°C compared with 32°C ( $F_{1,37}=30.3$ ,  $P<0.001$ ; Fig. 3A, inset). Temperature had no effect on the efficiency with which nutrients were either extracted from wheat or subsequently converted to biomass (Fig. 3B), and locusts were bigger at 38°C than at 32°C ( $F_{1,38}=14.68$ ,  $P<0.001$ ; Fig. 3C).

### Experiment 2: temperature selection following a meal of either wheat or kangaroo grass by either protein- or carbohydrate-deprived locusts

For locusts reared on 21P:21C for the first 72 h of the fifth stadium, following the manipulation of the locust's nutritional state, all nymphs feeding on wheat, irrespective of state (protein or carbohydrate deprived), selected an average temperature of  $37.8\pm0.2^\circ\text{C}$  ( $\chi^2=0.06$ , d.f.=4,  $P=0.861$ ; Fig. 4A, Table 1), with over 85% of insects selecting temperatures within the 38 or 41°C 'zones' (Fig. 4A). In contrast, locusts fed kangaroo grass chose the temperature at which they were most efficient at gaining from that grass the nutrient for which they were specifically deficient (Fig. 2A). Protein- and carbohydrate-deprived insects selected significantly different temperatures ( $38.3\pm0.4$  and  $34.1\pm0.4^\circ\text{C}$ , respectively;  $F_{1,38}=37.24$ ,  $P<0.001$ ; Fig. 4B). Eighty-five per cent of protein-deprived locusts selected 38 or 41°C (Fig. 4B) – the temperature zone at which more protein than carbohydrate is extracted from kangaroo grass (Fig. 2A). In contrast, only 20% of carbohydrate-deprived locusts selected these higher temperatures. However, fewer than 50% selected 32°C (Fig. 4B), at which they would have obtained more carbohydrate than protein (Fig. 2A) and therefore redressed their nutritional imbalance.

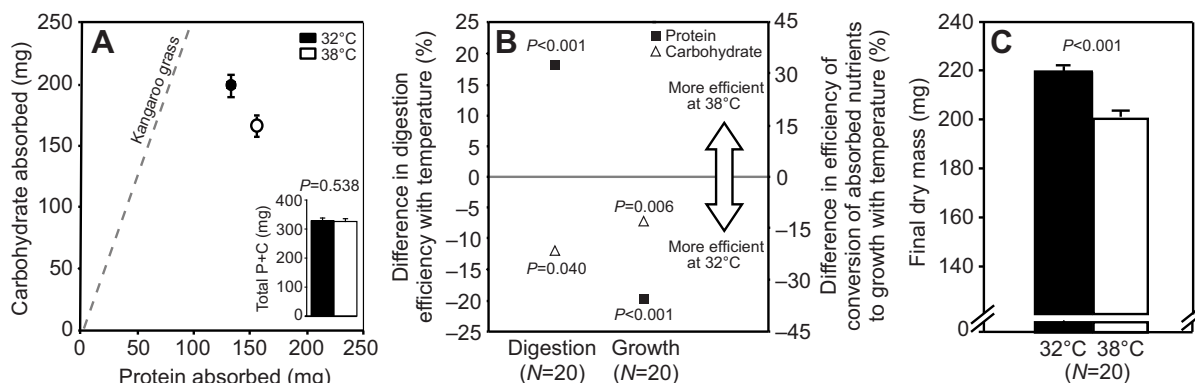


Fig. 2. (A) Nymphs fed kangaroo grass absorbed the same amount of protein (P) plus carbohydrate (C) at both 32 and 38°C ( $F_{1,37}=0.39$ ,  $P=0.580$ ; inset). However, at 32°C absorbed energy contained more carbohydrate than protein compared with 38°C (P:  $F_{1,37}=36.96$ ,  $P<0.001$ ; C:  $F_{1,37}=7.14$ ,  $P=0.011$ ; P:C:  $F_{1,37}=4.77$ ,  $P=0.035$ ). The grey dashed line gives the ratio of P:C for kangaroo grass. (B) Nymphs absorbed carbohydrates more efficiently at 32°C and protein more efficiently at 38°C, and both protein and carbohydrate growth was more efficient at 32°C. (C) Locusts feeding on kangaroo grass followed the temperature–size rule, with locusts reared at 32°C being bigger than those reared at 38°C ( $F_{1,38}=23.44$ ,  $P<0.001$ ). Values shown are means  $\pm$  s.e.m.; where s.e.m. are not visible they are contained within the symbol.

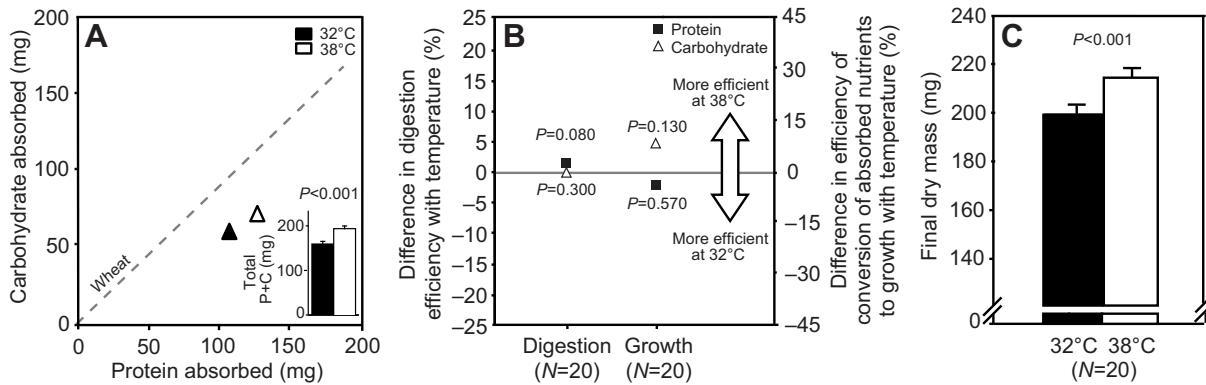


Fig. 3. (A) Locusts fed wheat obtained more energy (P+C) when feeding at 38°C than at 32°C (inset;  $F_{1,37}=30.28$ ,  $P<0.001$ ), but the ratio of P:C absorbed was not altered by temperature ( $F_{1,37}=1.28$ ,  $P=0.266$ ). (B) Both protein and carbohydrate were absorbed and converted to biomass with equal efficiency at both temperatures. (C) This pattern resulted in locusts showing temperature–size reversal on wheat, with larger final body sizes attained at 38°C than at 32°C ( $F_{1,38}=14.68$ ,  $P<0.001$ ). Values shown are means  $\pm$  s.e.m.; where s.e.m. are not visible they are contained within the symbol.

### Experiment 3: can temperature selection performance be improved with experience?

Following the 3 day period where locusts were able to experience the nutritional outcomes when ingesting kangaroo grass at both temperatures, before being rendered protein or carbohydrate deprived, the capacity of carbohydrate-deprived insects ingesting kangaroo grass to choose 32°C improved substantially (Table 2, Fig. 4B,C), with over 85% of locusts now choosing 32°C – a 70% improvement from that of naive locusts. Protein-deprived locusts ingesting kangaroo grass also improved marginally, from an 85% success rate at choosing 38°C without having previously ingested kangaroo grass to 95% after experience (Table 2). The temperature regime (starting at either 32 or 38°C) experienced during the rearing period from Day 0 to 3 had no effect ( $P>0.5$ ) on temperature selection and this term was removed from the analysis.

### DISCUSSION

Although temperature-specific nutrient digestion has been recorded in other ectotherms (Bendiksen et al., 2003; Zhang et al., 2009), and ectotherms are known to use temperature to ameliorate the effects of starvation (Angilletta, 2009; Coggan et al., 2011), to our knowledge this is the first example of an ectotherm using temperature selection to redress specific nutrient imbalances. Additionally, locusts improved their ability to address nutrient imbalances by temperature selection following experience. This provides a rare example of a learned nutrient-specific regulatory response. The discovery that an insect is able to select temperatures to meet the specific nutritional challenges posed by different host plants has implications for interpreting host-plant choice and has consequences for our understanding of how changed environmental temperatures may affect animal–plant interactions.

For locusts feeding on both grasses, the differences in mass with temperature were primarily a consequence of differences in the digestion and absorption of protein and carbohydrate and secondarily a consequence of losses due to post-absorptive metabolism (Figs 1–3). When feeding on synthetic diets, regardless of temperature or the ratio of protein and carbohydrate, both nutrients were absorbed with equal efficiency (Miller et al., 2009). When ingesting intact leaves, locusts are unable to absorb all the ingested protein and carbohydrate (reviewed by Clissold, 2007) and we found the digestion and absorption of protein and carbohydrate occurred in a host-plant-specific way with temperature (Fig. 2A, Fig. 3A).

For locusts feeding on wheat, temperature did not alter the percentage of protein and carbohydrate absorbed nor allocated to body mass (Fig. 3B). Rather, intake rate increased with increasing temperature, and as a consequence locusts absorbed ca. 30% more energy (protein + carbohydrate) at 38°C than at 32°C (i.e. Fig. 1Bi vs Biii). This contrasts with our previous results where we found temperature had a profound effect on nutrient utilization efficiency of synthetic diets, with locusts reared at 38°C retaining substantially less of absorbed energy than those reared at 32°C (Miller et al., 2009). At 32°C, locusts obtained nutrients from wheat at a much reduced rate, which would increase metabolic costs (Clissold et al., 2009), potentially nullifying the improved nutrient utilization previously recorded at 32°C.

Although a difference in the total energy absorbed due to differences in intake rates of total energy (protein plus carbohydrate) explains differences in body mass with temperature for locusts feeding on wheat, this was not the case for locusts feeding on kangaroo grass. Similar amounts of kangaroo grass were ingested by locusts at both temperatures, but protein was absorbed with greater efficiency at 38°C and carbohydrate with greater efficiency at 32°C (Fig. 2B, Fig. 1Bi vs Bii). This resulted in a lower ratio of P:C being absorbed at 32°C than at 38°C (Fig. 2A). Increased body mass when eating diets in which carbohydrate is oversupplied relative to protein has previously been recorded for *L. migratoria* (Raubenheimer and Simpson, 1999). The much poorer efficiency with which protein was retained as body mass at 38°C was fully consistent with previous work when locusts are ingesting synthetic diets high in protein (Raubenheimer and Simpson, 1999). Previous research has shown that locusts are able to use a variety of post-absorptive mechanisms (Fig. 1) to maintain homeostasis when ingesting protein and carbohydrate in sub-optimal ratios (Raubenheimer and Simpson, 1999).

Among ectotherms, size at maturity is typically reduced with increasing temperatures, a form of phenotypic plasticity known as the temperature–size rule (TSR) (Atkinson, 1994). The robustness of this association has been challenged, as the TSR can be readily reversed by altering diet quality (Berrigan and Charnov, 1994; Diamond and Kingsolver, 2010; Kingsolver and Huey, 2008; Mousseau, 1997; Walters and Hassall, 2006). ‘Poor’ diet is often provided as an explanation of TSR reversal (e.g. Diamond and Kingsolver, 2010). Locusts feeding on wheat are such an example

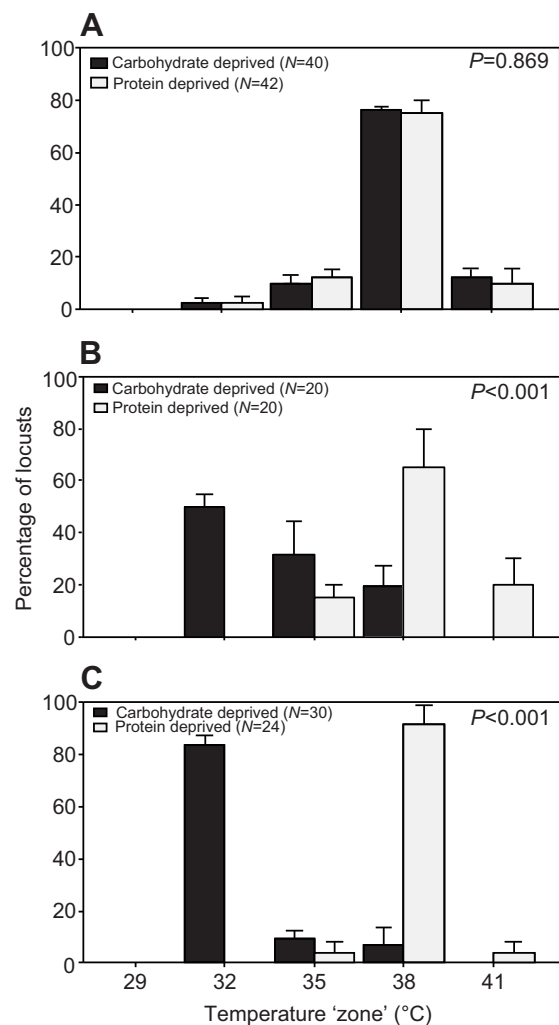


Fig. 4. The percentage of locusts in either a carbohydrate- or protein-deprived state (black or grey bars, respectively) selecting a particular temperature 'zone' when placed in a thermal gradient following a meal of grass. (A) Almost all (>85%) of the locusts fed wheat selected 38 or 41 °C regardless of nutritional state. (B) Following a meal of kangaroo grass, temperature selection by nymphs was dependent on nutritional state, with 85% of protein-deprived locusts selecting temperatures greater than or equal to 38 °C and ~50% of carbohydrate-deprived locusts selecting 32 °C. (C) Following a period of time where locusts could experience ingesting kangaroo grass at either temperature, locusts became near faultless in choosing the temperature that best redressed nutritional imbalances. Values shown are means  $\pm$  s.e.m.; *P*-values compare the temperature selection of protein- and carbohydrate-deprived locusts ( $\chi^2$  statistic).

of TSR reversal, with locusts feeding on kangaroo grass following the typical TSR. In neither case is diet 'quality' a useful or sufficient explanation. Rather, by determining protein and carbohydrate ingested, absorbed and subsequent allocation to growth, we have been able to describe the nutritional underpinning of growth differences with diet and temperature for *L. migratoria* when feeding on these two grasses. Further research is required to understand the mechanisms responsible for these nutritional differences, before general predictions regarding the effects of temperature on insect herbivores can be made. Such mechanisms could include differential changes in the activities of enzymes and transporters, and interactions with secondary metabolites (Angilletta, 2009; Stamp, 1990; Stamp and Yang, 1996). Regardless of the mechanism,

Table 1. Results of ANOVA for the effects of grass species (kangaroo grass and wheat) and nutrient state (protein or carbohydrate deprived) on temperature selection (average) by locusts following a meal of either grass

Source of variation	d.f.	MS	F	P
Grass species	1	65.74	17.07	<b>&lt;0.001</b>
Nutrient state	1	118.14	30.68	<b>&lt;0.001</b>
Grass species $\times$ Nutrient state	1	109.83	28.52	<b>&lt;0.001</b>
Residual	109	3.85		

Significant *P*-values (<0.05) are highlighted in bold.

temperature altered the ratio of protein and carbohydrate absorbed from kangaroo grass and thus offered locusts the opportunity to select a temperature and prioritize optimizing nutrient uptake and maximizing body mass or prioritize development rate at the expense of growth.

Rates of both growth and development were maximized for locusts feeding on wheat at 38 °C (Fig. 3), and they selected 38 °C or higher regardless of deprivation state following a meal of wheat (Fig. 4A). However, locusts fed kangaroo grass adopted a temperature consistent with optimizing the gain of the nutrient that was in shortage (Fig. 4). That is, locusts used temperature choice to correct a nutritional imbalance (Fig. 2). Previously it has been demonstrated that this same locust species is able to associate odour (Simpson and White, 1990) and colour (Raubenheimer and Tucker, 1997) cues with the protein and carbohydrate content of artificial diets, and to be attracted by the appropriate cue only when in a specific state of nutrient imbalance (Raubenheimer and Tucker, 1997). In such cases, the subsequent redressing of the nutritional imbalance occurs upon eating the food associated with the cue. Here we have shown, for the first time in any animal species as far as we are aware, that locusts associate temperature cues not with the presence of a nutritionally appropriate food, but with differential effects of temperature on digestion of nutrients from an already ingested meal.

Many insects, including grasshoppers and locusts, can rapidly associate specific nutrients with olfactory or visual cues (Dukas, 2008), and such learning has been shown to allow a grasshopper, *Schistocerca americana*, to achieve higher growth rates in a laboratory setting (Dukas and Bernays, 2000). However, when feeding on kangaroo grass and carbohydrate deprived, locust temperature choice favoured maximizing body size at maturity and compromising rates of development. Life history outcomes and subsequent fitness of insects are strongly influenced both directly and indirectly by rates of development and final size at maturity (e.g. H  ggstr  m and Larsson, 1995; Roff, 1992; Rowe and Ludwig, 1991; Stamp, 1990). Prolonged juvenile development has been associated with time-dependent mortality risks and the inability of

Table 2. Results of ANOVA for the effects of knowledge state (naive or experienced) and nutrient state (protein or carbohydrate deprived) on temperature selection (average) by locusts following a meal of kangaroo grass

Source of variation	d.f.	MS	F	P
Knowledge state	1	14.79	4.81	<b>0.031</b>
Nutrient state	1	501.59	162.98	<b>&lt;0.001</b>
Knowledge state $\times$ Nutrient state	1	12.28	3.99	<b>0.049</b>
Residual	89	3.08		

Significant *P*-values (<0.05) are highlighted in bold.



insects to exploit temporally short resources (e.g. Creel and Christianson, 2008; Feeny, 1970). But it has been demonstrated that learning can be beneficial in optimising performance in heterogeneous environments, especially when dealing with features unique to a location and time (Dukas, 2008; Dukas and Bernays, 2000). Insects typically have rapid rates of development, and making use of experience to capitalize on the fine-scale thermal variation present in the natural environment (Willmer, 1982) may decrease risks associated with moving between food patches when foraging (Bernays, 1997; Gotthard, 2000), or allow insects that specialise on single host plants to meet nutritional requirements as the nutritional quality of leaves change over time (e.g. Read et al., 2003). Field studies are required to understand how locusts balance requirements for growth (optimizing nutritional needs) and rates of development given temperature and the available host plants.

The discovery that an insect is able to select temperatures to meet the specific nutritional challenges posed by different host plants highlights the pitfalls of trying to interpret an animal's preferences for foods or temperatures. Recent evidence suggests that the probability of a leaf being eaten depends on a complex suite of plant and herbivore traits (Agrawal, 2011; Carmona et al., 2011) and we suggest also abiotic conditions. As small ectotherms have abundant opportunities to make adaptive adjustments to body temperature by small-scale habitat selection (Willmer, 1982), host-plant use must be considered in a temperature context. Therefore, micro-climatic effects may be of more importance than previously appreciated in predicting the ecological consequences of climate change and altered land-use practices (Kearney et al., 2009; Suggitt et al., 2011).

## APPENDIX

### Methods: rendering locusts either protein or carbohydrate deprived

The aim was to rapidly make the locusts either protein or carbohydrate deprived. While the treatment diets have rather extreme ratios, they were offered to the locusts over the shortest period of time possible so that ca. 95% of the locusts would subsequently select foods containing higher amounts of the nutrient in which they were deficient. To confirm that locusts were rendered protein or carbohydrate deprived by the 4 h treatment on unbalanced synthetic food, 20 locusts, not otherwise used for experiments, were randomly selected in a design balanced by experiment and rearing conditions, and placed individually in clear plastic containers (11×17×5 cm, length × width × height) with water and two small feeding bowls, one containing food high in protein, 35P:7C, and the other containing food high in carbohydrate, 7P:35C. Behaviour was videoed and the first meal to be eaten was scored.

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## AUTHOR CONTRIBUTIONS

F.J.C. designed, performed and analysed the experiments and was the primary writer. N.C. assisted with the data collection. S.J.S. contributed to the writing of the manuscript.

## COMPETING INTERESTS

No competing interests declared.

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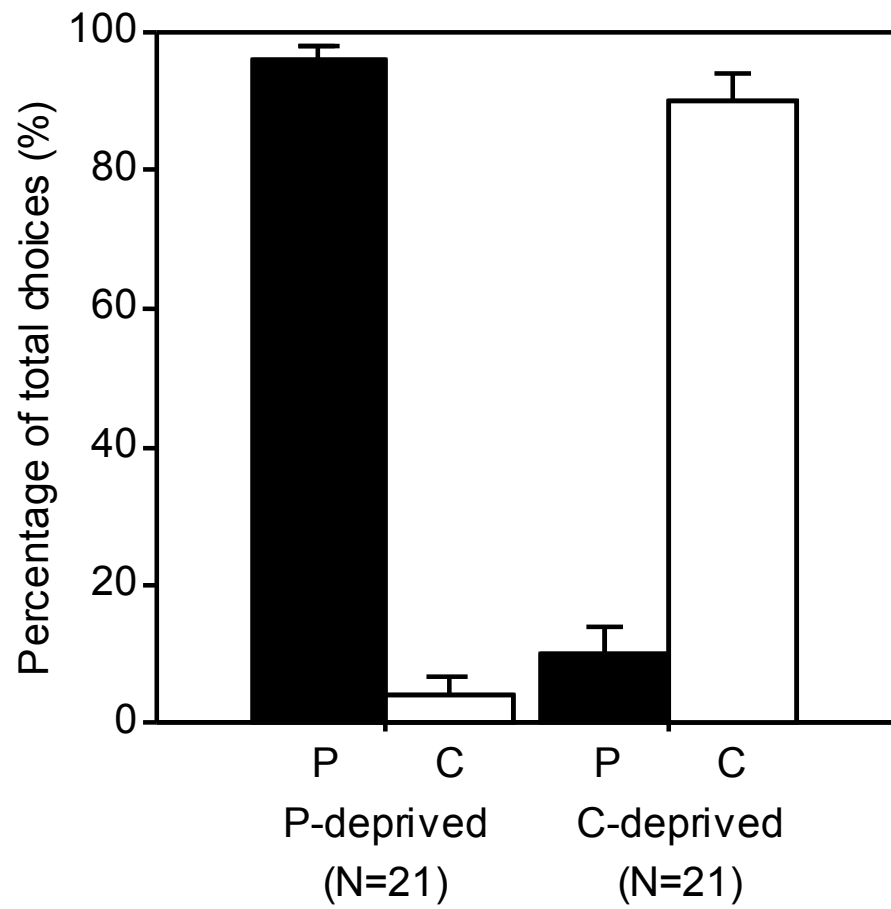


Fig. S1. When given a choice between a food high in protein (35P:7C) or one high in carbohydrate (7P:35C), locusts in either a carbohydrate- or protein-deprived state selected the nutritional complement.

**Table S1.** Chemistry of both grasses over the duration of the experiment. Replicates are days (n= 9) with all parameters determined from five separate samples per day. Values given are for means  $\pm$  SEM and *P* values from ANOVA.

	<i>T. triandra</i>	<i>P</i>	<i>T. aestivum</i>	<i>P</i>
<i>Parameter</i>				
Water (g.g <sup>-1</sup> dry matter)	1.6 $\pm$ 0.02	0.338	7.8 $\pm$ 0.06	0.361
Protein	7.9 $\pm$ 0.4	0.124	13.4 $\pm$ 0.5	0.102
Non-structural carbohydrate	23.7 $\pm$ 0.5	0.733	12.8 $\pm$ 0.4	0.571
Ratio P:C	1:2.9	0.230	1:0.9	0.137

**Table S2.** Water loss and metabolic changes of the grass blades after 24h at 32°C and 38°C. Metabolic changes, in terms of total dry mass, protein and carbohydrate were quantified after Bowers et al. (1991) except that the dry mass initially was calculated from a regression of fresh mass against dry mass for a known sample rather than a ratio of fresh mass/dry mass. The values are expressed relative to that predicted from a subset of grasses taken initially (time = 0). A value of 1 indicates no change, while a value greater than 1 indicates the leaf material has gained dry mass (or protein or carbohydrate) compared to that initially predicted, and values less than 1 indicate that there has been a loss. Values given are means  $\pm$  SEM and *P* values are from ANOVA.

<i>Room temperature</i>	<b>32°C</b>	<i>P</i>	<b>38°C</b>	<i>P</i>
<b><i>T. triandra</i></b>				
<i>Parameter</i>				
Water	0.97 $\pm$ 0.02	0.154	0.95 $\pm$ 0.02	0.137
Total dry matter	1.02 $\pm$ 0.02	0.406	1.00 $\pm$ 0.01	0.865
Protein	0.96 $\pm$ 0.03	0.215	0.96 $\pm$ 0.04	0.352
Non-structural carbohydrate	1.01 $\pm$ 0.03	0.810	0.97 $\pm$ 0.02	0.251
<b><i>T. aestivum</i></b>				
<i>Parameter</i>				
Water	0.97 $\pm$ 0.07	0.063	0.71 $\pm$ 0.13	0.080
Total dry matter	1.00 $\pm$ 0.03	0.965	0.96 $\pm$ 0.03	0.069
Protein	0.99 $\pm$ 0.04	1.00	0.99 $\pm$ 0.04	0.391
Non-structural carbohydrate	0.99 $\pm$ 0.06	0.799	0.99 $\pm$ 0.07	0.215

### ***Reference***

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