Locusts use dynamic thermoregulatory behaviour to optimize nutritional outcomes

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Because key nutritional processes differ in their thermal optima, ectotherms may use temperature selection to optimize performance in changing nutritional environments. Such behaviour would be especially advantageous to small terrestrial animals, which have low thermal inertia and often have access to a wide range of environmental temperatures over small distances. Using the locust, Locusta migratoria, we have demonstrated a direct link between nutritional state and thermoregulatory behaviour. When faced with chronic restrictions to the supply of nutrients, locusts selected increasingly lower temperatures within a gradient, thereby maximizing nutrient use efficiency at the cost of slower growth. Over the shorter term, when locusts were unable to find a meal in the normal course of ad libitum feeding, they immediately adjusted their thermoregulatory behaviour, selecting a lower temperature at which assimilation efficiency was maximal. Thus, locusts use fine-scale patterns of movement and temperature selection to adjust for reduced nutrient supply and thereby ameliorate associated life-history consequences.

Keywords: dynamic thermoregulatory behaviour; nutrition; phenotypic plasticity; life-history trade-offs

1. INTRODUCTION

Ectotherms thermoregulate to buffer the effects of environmental heterogeneity; however, no single body temperature will simultaneously optimize all metabolic processes. This is because different processes respond differently to temperature and have different thermal optima [1–5]. As a result, in response to biological and ecological challenges, ectotherms often prioritize the function of one process at the expense of others by varying their body temperatures. For example, reptiles and insects increase body temperature in response to pathogens [6–9], or to enable efficient locomotion (e.g. [4,10]). Conversely, selecting lowered body temperatures has been associated with increased survival of hosts when attacked by pathogens through altered immune function as well as the direct effect of temperature on the pathogen itself [11].

Besides temperature, the other major determinant of metabolism is nutrition. Nutrient availability mediates the effect of temperature on growth and development [12,13]. Gross rates at which nutrients are assimilated into growth rise with increasing temperature in ectotherms until a critical temperature, beyond which net nutrient assimilation declines because the conversion of absorbed food to biomass becomes energetically inefficient (e.g. fishes [14]; reptiles [5,15,16]; insects [5,12,13]). Thus, many ectotherms when starved select lower body temperatures as a means to conserve limiting energy (e.g. [17]).

Recently, Miller et al. [13] found that locusts reared at 32°C or 38°C grew to the same size and body composition, but those at the lower temperature had higher nutrient assimilation efficiency and grew more slowly than at the higher temperature. When offered a choice of temperatures in a thermal gradient, locusts selected 38°C, thereby prioritising growth rate over assimilation efficiency. In the experiment of Miller et al. [13], high-quality food was present in excess throughout the study. The question arises whether the insects would have prioritized growth rate over utilization efficiency had food been limited, either in quality or quantity. Under such conditions, locusts could make the most of ingested food and ameliorate the growth costs of nutrient limitation by choosing a temperature closer to 32°C than to 38°C. Locusts and other insects are well suited to use such a strategy since, being small, they both have low thermal inertia, allowing rapid changes in body temperature, and typically have access to a wide choice of temperatures and microclimates over small distances [18,19].

Reductions in the supply of nutrients occur at two time scales. In the short term, animals impose periods of reduced supply on themselves during the normal course of feeding, in which bouts of feeding are followed by post-prandial periods without ingestion. Over the longer term, nutrient supply may be compromised by having limited access to food or by being confined to nutritionally unbalanced or nutritionally dilute foods. Our aim in the present study was to discover whether locusts could respond to immediate and/or chronic reductions to their nutrient supply by selecting a lower temperature, at which assimilation efficiency but not growth rate is favoured, than when high-quality food is abundant.

2. MATERIAL AND METHODS

(a) Locusts

Gregarious stocks of Locusta migratoria originally collected from the Central Highlands of Queensland, Australia, were reared at The University of Sydney. Locusts were reared at a density of 500–1000 individuals on seedling wheatgrass and wheat germ in large plastic bins (56 × 76 × 60 cm) under a 14 L:10 D photoperiod in a room kept at 32°C. During the ‘light-on’ phase, each bin had an additional...
heat source (250 W heat lamp) mounted on the mesh roof of the bin.

(b) Thermal gradient arenas

Thermal gradients were established on steel plates (six), 700 mm long × 100 mm wide and 10 mm deep, by placing one end of the plate on an ice bath and a 150 W ceramic heat lamp beneath the other end of the plate. Locusts were confined to a region on the plate between 18°C and 58°C by using an elliptical 300 mm high wall coated in Fluon (liquid Teflon) to prevent climbing. The thermal gradient plate and walls were painted white to provide visual contrast, allowing locust movement to be quantified using video analysis. Contrast was also enhanced by the use of fluorescent tubes (16 W) located 1 m above each arena. The minimum and maximum temperatures along the plate were set to include known thermal limits for activity in locusts, which do not exceed 53°C and are rarely less than 28°C [20,21].

The thermal profile of each plate was determined using infrared images (IR Camera S65, FLIR Systems) and measurements obtained using a 0.5 mm beaded wire K type thermocouple (YC-747D, Yu Ching Technology, Taiwan). Thermocouple measurements were also taken 0.5 and 1 cm (the height of the thorax of a fifth instar nymph) above the plate at 3°C intervals. Although each plate varied slightly, a linear 0.6°C cm⁻¹ change in temperature occurred between ca 27°C and 45°C (electronic supplementary material, figure S1), with the temperature being the same at and 1 cm above the surface of each plate. The heated and cooled ends of each plate were reversed between adjacent plates to control for the effects of position in the room.

(c) Experimental conditions

(i) Post-prandial patterns

Freshly moulted (within 4 h of ecdisis, termed day 0) fifth instar male nymphs whose initial fresh weight was between 410 and 455 mg were housed individually until day 3 in clear plastic cages (17 (l) × 12 (w) × 6 (h) cm) containing water, a metal perch and abundant food. The conditions were the same as for the stock culture except that no additional source of heat was provided. On day 3, locusts were provided with a meal of seedling wheatgrass, and once observed to have ingested a meal, the locust was placed at a random position on a thermal gradient plate and its behaviour was recorded for 90 min. A meal was defined as eating for a minimum of 2 min and not feeding again within 4 min [22]. Locust behaviour was captured on digital video (JVC Everio) and then quantified using ETHOVISION XT (Nodus IT) and infrared images (IR Camera S65, FLIR Systems). Increments of 3°C were chosen as these are well within a locust’s sensory detection capacity [23,24]. Additionally, it was found that locusts only moved within a temperature range spanning 27–45°C, and across this region of the plate, each 3°C zone was ca 5 cm long, which was greater than the length of the locust (ca 3 cm). If locusts spanned two adjacent zones, the location of the thorax of the locust was used as the point of reference. The middle of each 3°C zone was recorded as the temperature for a zone; i.e. a locust in the zone 36.5–39.5°C was said to be at 38°C. Locusts were identified and tracked in ETHOVISION using the grey scale method. To prevent small body movements from being calculated as locomotion, the distance moved was smoothed by setting 20 per cent lower and 60 per cent upper thresholds for mobility. Recorded behaviour was integrated over 5 min intervals and three behaviours were quantified: (i) time spent in each temperature zone, (ii) time spent moving, and (iii) distance moved. The speed with which the locust moved was derived from the distance moved and time spent moving.

When placed on the thermal gradient plates following a meal, locusts spent periods of time locomoting and settling, with the positions of the latter being used to infer temperature preferenda. To establish baseline measures of behaviour on the plate in the absence of a temperature gradient, two control trials were run in which the thermal gradient plates were set at a constant 32°C or 38°C, the two temperatures at which locusts were found to settle in the presence of a gradient. Uniform temperatures along the thermal gradient plate were created using two 150 W ceramic heat lamps beneath the plates.

To determine whether the pattern of temperature selection found in the presence of a gradient was a direct response to feeding, locusts were placed on the thermal gradient plate following a meal as described above. After 55 min (an average intermeal interval on wheat during ad libitum feeding [22]), the locusts were provided with a vial (5 ml florist’s vial) containing either fresh wheatgrass or nothing. All vials were placed midway between 32°C and 38°C. After 15 min, all vials and any food were removed from the arenas and behaviour was recorded for another 35 min. All locusts offered wheatgrass ingested a meal. All trials were randomized over time and the behaviour of six locusts was recorded per treatment.

(ii) Temperature selection in response to diet ‘quality’

Freshly moulted (within 4 h of ecdisis) fifth instar male nymphs were housed as described above but rather than being provided with seedling wheat, locusts were provided with one of five dry granular synthetic foods containing an equal ratio of protein (P) and carbohydrate (C) but varying in the total concentration (P + C) [25]. The concentrations were P + C = 42, 38, 34, 30 and 15 per cent, with all diets containing 4 per cent salts, vitamins and sterols and the remainder consisting of indigestible cellulose. Rates of growth and development are optimal for locusts feeding on a diet where P and C are close to a 1 : 1 ratio at a total concentration of 42 per cent [26]. The behaviour of six locusts was recorded per treatment, except for P + C = 30, in which 12 locusts were recorded. All treatments were randomized over time.

(d) Data analysis

Speed and distance moved were modelled separately (repeated-measures ANOVA) using a partly nested design (time within insect) with diet treatment as the between-subject factor. One-sample, one-tailed t-tests at each time were used to determine when the animal was stationary (testing whether the mean was greater than 0). It was apparent that the majority of insects were stationary during two time periods after feeding (20–45 min and 70–90 min), and the temperature selected at these two times was investigated using a repeated-measures design followed by ANOVA to compare the selected temperatures at each time separately. A Spearman’s rank correlation coefficient was used to compare the relationship between nutrient intake and temperature selected. Box plots were used to check for normality and homogeneity of variances across the treatments prior to analysis. All analyses were conducted in SYSTAT v. 12 (Systat Software, Chicago, IL, USA).

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3. RESULTS

(a) Post-prandial behaviour

Across the 90 min period, post-feeding locomotion shifted between periods of movement and periods during which locusts remained relatively stationary (figure 1). This was the case whether the locusts were on a plate with a temperature gradient or one where the temperature was constant. Additionally, this pattern of movement was the same whether or not a meal was ingested 55 min after being placed on the thermogradient plates (figure 1). Four periods were identified following the ingestion of a meal: (i) from 0 to 20 min locusts spent greater than 50 per cent of the time moving around ‘exploring’ the thermal gradient plate at an average speed of 1.5 cm min$^{-1}$; (ii) from 20 to 45 min locusts moved for less than 20 per cent in each 5 min interval at a mean speed of 0.68 ± 0.25 cm min$^{-1}$, moving on average 0.63 ± 0.27 cm, i.e. they typically stayed within a temperature zone; (iii) from 45 to 70 min the locusts increased in activity, moving at an average speed of 2.11 ± 0.29 cm min$^{-1}$; and (iv) from 70 to 90 min locusts were again near stationary, moving on average at a speed of 0.19 ± 0.19 cm min$^{-1}$ and traveling 0.25 ± 0.20 cm. If food deprivation continued beyond 90 min, locusts became increasingly active and abandoned thermoregulation (data not shown).

Locusts confined to thermal plates where the temperature was either a constant 32°C or 38°C demonstrated the same pattern of movement following a meal ($F_{1,184} = 0.12$, $p = 0.857$); however, locusts on the 32°C plate walked about eight times faster and went almost four times further than those on the 38°C plate (speed: $F_{1,184} = 27.98$, $p = 0.001$; distance: $F_{1,184} = 58.84$, $p = 0.001$). When on the constant-temperature plates, locusts showed no evidence of being in any particular position along the plate (determined as distance from one end; $F_{1,20} = 0.20$, $p = 0.670$). Thus, during the two time intervals in which the locusts were stationary on the thermal gradient plates (20–45 and 70–90 min), it was deemed they were demonstrating a temperature selection preference.

Following a meal, locusts selected 38 ± 0.5°C to settle for the first stationary period (figure 2). If locusts were not fed again, their subsequent movement was directed towards a lower temperature, coming to rest for the next stationary period at 32.0 ± 0.8°C (figure 2b).

Figure 1. (a,b) Patterns of speed and (c,d) distance moved for locusts following a meal of seedling wheatgrass, where (a,c) no further meals were provided for the following 90 min and (b,d) where another meal of seedling wheatgrass was provided at 55 min (n = 6). Solid horizontal arrows show the times where locusts were stationary, i.e. movement was within a temperature zone, and dashed horizontal arrows where locusts were moving between temperature zones. Behaviour was not recorded for 15 min (as indicated by the break) while locusts consumed a second meal 55 min after the completion of the first meal. All trials were randomized over time and the behaviour of six locusts was recorded per treatment.

Figure 2. Contour plot showing the percentage of locusts recorded in each 3°C temperature bin, following an initial meal and then if a second meal was ingested (a) or not (b). All trials were randomized over time and the behaviour of six locusts was recorded per treatment.
Table 1. Results for the comparison of locusts that fed after 55 min and those that did not. (Significant $p$-values ($p < 0.05$) are highlighted in bold.)

<table>
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<tr>
<td>distance moved</td>
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<td>0.42</td>
<td>0.10</td>
<td>0.754</td>
</tr>
<tr>
<td></td>
<td>locust (treatment)</td>
<td>10</td>
<td>4.91</td>
<td>1.45</td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>18</td>
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<td>2.37</td>
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<td>3.29</td>
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<tr>
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However, if a meal was consumed 55 min after the initial meal, all locusts returned to 38.0 ± 0°C rather than continuing to move towards a cooler temperature (figure 2a) (paired $t$-test: $t_5 = 7.91$, $p = 0.001$). Neither the speed nor distance travelled differed between feeding treatments (table 1), just the temperature they moved to.

(b) Response to diet quality

The post-prandial pattern of locomotion, switching between periods of movement or relative immobility, was demonstrated by all locusts feeding on diets that ranged in concentration from 42 to 34 per cent ($P + C$), but by only $ca$ 60 per cent of the locusts feeding on 15P : 15C and by none of the locusts feeding on the most dilute diet ($P + C = 15\%$) ($F_{4,245} = 3.69$, $p = 0.015$, pairwise post hoc tests; $p < 0.05$ for locusts ingesting $P + C = 15\%$ compared with all other diets, for all other post hoc comparisons $p > 0.05$; figure 3). Dilution of the diets reduced nutrient supply significantly through failure of locusts to compensate fully by increasing food intake ($F_{4,245} = 368.14$, $p < 0.001$; pairwise post hoc tests all $p < 0.05$). There was a significant negative log–log relationship between the amount of nutrients consumed in the 24 h prior to the experiment and movement from 20 to 25 min following the ingestion of a meal ($\log\text{(movement)} = -3.88 \log\text{(P+C eaten)} + 16.71$; $F_{1,16} = 17.22$, $p = 0.001$). For locusts that were quiescent 25 min following the ingestion of a meal, treatment diet affected temperature selection ($F_{3,20} = 35.26$, $p < 0.001$). The temperature selected by locusts after feeding correlated positively with the amount of $P + C$ ingested in the preceding 24 h (Spearman’s rank-order correlation: $r_{23} = 0.84$, $p < 0.001$, $\rho^2 = 0.84$; figure 4). All locusts ingesting less than $ca$ 45 mg ($P + C$) but more than $ca$ 37 mg in the previous 24 h selected 32° C and all locusts ingesting more than $ca$ 60 mg ($P + C$) selected 38° C. Thus, as the diet ingested decreased in ‘quality’ owing to dilution of nutrients resulting in grass-hoppers ingesting less nutrients, a temperature closer to 32° C was selected.

4. DISCUSSION

Locusts with uninterrupted access to food that was near nutritionally optimal for their requirements selected a temperature (38° C) at which rates of growth and development were previously found to be high [13]. However, when faced with reductions to their food supply in the short term, by not being able to find the next meal in the normal course of feeding, locusts selected a lower temperature (32° C), at which the conversion of nutrients to biomass is favoured [13]. With increasing reductions to the supply of nutrients over longer time intervals via nutrient dilution, locusts selected an increasingly lower temperature from 38° to 32° C, providing further evidence that they use thermoregulatory behaviour to maximize the growth gained from ingested nutrients.

Previously, many ectotherms have been observed in the field to move to lower temperatures after feeding (e.g. [27–30]). These post-prandial patterns of movement are thought to be thermoregulatory, enabling animals to conserve energy through a reduction in metabolic rate. However, to date, evidence for a potential role of ther-

Figure 3. Locust locomotory behaviour recorded from 20 to 25 min following the ingestion of a meal where the concentration of nutrients ($P + C$) ranged from 15 to 42%. All locusts feeding on diets that ranged in concentration from 42 to 34% ($P + C$) ceased movement 20 min following a meal. However, $ca$ 40% of the locusts feeding on $P + C = 30\%$ and all of the locusts feeding on the most dilute diet ($P + C = 15\%$) continued moving, abandoning thermoregulatory behaviour.
the average intermeal interval (54 min in our study closely matched the feeding behaviour and the alternation between resting and locomotory behaviour: motion and ‘searching’ for the next meal. The timing of while processing ingesta, before again commencing locomotion periodically. After a meal, they are relatively quiescent for searching for food (figure 1). This second resting period typically lasts ca 20 min before locomotion recommences and is most likely the result of an endogenous intermediate length oscillation underling feeding and locomotory behaviours [41]. By contrast, when locusts were faced with extreme nutrient shortage (figure 3), the typical oscillation between rest and movement was abandoned in favour of continuous locomotion, as has been observed previously [42].

Despite net rates of nutrient assimilation being lower at 38°C, if locusts were able to maintain metabolizable nutrient intake above ca 60 mg d⁻¹, they selected 38°C, at which development rate is high [13]. For many juveniles, life-history outcomes and subsequent fitness are strongly influenced by time constraints imposed by seasonality [43,44]. Rapid growth and rates of development can be favourable to lifetime fecundity for insects, as time-dependent mortality risks, such as attack from pathogens, predators and disease, are minimized [45–47]. In addition, rapid development allows the exploitation of short-lived nutritional resources [48,49] and many ectotherms can benefit from being at a higher temperature through increased resistance to the effects of pathogens, parasites and diseases [50–52].

For developing juveniles, any decrease in the net rate at which nutrients are assimilated can reduce fitness through the increased time it takes to reach maturity and/or decreased adult size (e.g. [53–57]). Reducing overall metabolic costs by decreasing body temperature allows locusts to allocate a greater proportion of absorbed nutrients to growth, although development time will be prolonged [13]. If locusts remained at 38°C but were unable to fully supply nutrient requirements, they would have paid the double price of ending up smaller as an adult and taking longer to develop [58]. Locusts ingesting less than 60 mg metabolizable nutrients (P + C) in the previous 24 h increasingly selected lower temperatures at the rate of 1°C per 2.5 mg reduction in intake down to a minimum of 32°C. All locusts that ingested less than ca 45 mg metabolizable nutrients but more than 37 mg in the previous 24 h selected 32°C (figure 4). If intake fell below 37 mg, then locusts showed high rates of locomotion and did not settle at any particular temperature.

Our finding that locusts selected 38°C following the ingestion of a high-quality meal appears to conflict with field observations where animals have been observed to always select lower temperatures following feeding (e.g. [27–30]). However, this may be a consequence of the growth stage of the animal. Patch selection in the field has usually been recorded for mature animals, while our results are for developing juveniles. To fuel ontogenetic growth and ensure rapid development, juveniles have to maintain much higher intake rates than fully grown adults. For adults, the costs of slowed growth are less relevant, and therefore increasing the time between meals may be advantageous. Moving between patches, either to feed or thermoregulate, can be risky and costly; energy is expended moving to and from locations, movement itself can attract predators or scare off prey and feeding often attracts natural enemies more than when not feeding [1,59–61]. After feeding, an adult could therefore prolong the period between risky feeding bouts by moving to a lower temperature to process ingesta.

Body size and habitat (notably aquatic versus terrestrial) may limit the degree of flexibility ectotherms have to alter body temperatures throughout the day [62]. However, observed patterns of movement for many animals following feeding suggests that mid-sized ectotherms, especially

Figure 4. The relationship between the amount of protein (P) + carbohydrate (C) ingested in the preceding 24 h and temperature selection following the ingestion of a meal. Locusts that in the previous 24 h consumed more than ca 60 mg of a 1 : 1 mix of P + C selected 38°C to process ingesta; however, locusts that consumed less than ca 60 mg P + C selected a temperature increasingly closer to 32°C, with all locusts consuming less than ca 45 mg selecting 32°C. There was a strong correlation between selected temperature and the amount of P + C ingested (p < 0.001). Following the ingestion of a meal, the straight white line represents the amount of food ingested in the previous 24 h, where all locusts selected either 32°C or 38°C, and the wavy line where locusts varied which bin they ‘sat in’.
those in aquatic environments where heat loss/gain can be up to four times faster than on land, may also be using thermoregulatory behaviour over short time intervals to alter nutritional outcomes (e.g. [27–30]). Locusts, like many insects, are able to change the temperature of their body within seconds [19,63]. Insects are therefore able to easily move short distances between areas of differing temperatures, and it is likely that the benefits gained by insects from thermoregulation far outweigh the costs of moving to adjust body temperature [1,59–61,64].

Animals encounter temporal and spatial variation in resources, and foraging models typically assume that fitness is maximized in patches where gross intake rates are highest [65]. However, for ectotherms, nutrient acquisition is more than just a function of the available nutrients in a patch, and optimal patch choice may need to take into account the interplay between gross and net energy and nutrient uptake. Furthermore, mechanistic models linking functional traits with population-level outcomes (e.g. biophysical ecological [66] and dynamic energy budgets [67,68]) need to account for the degree to which an organism can use behaviour to modify physiological outcomes and thus be buffered from the effects of the prevailing conditions [69].

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