

RESEARCH ARTICLE

Effects of foraging distance on macronutrient balancing and performance in the German cockroach *Blattella germanica*

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ABSTRACT

The German cockroach (*Blattella germanica*) is an excellent model omnivore for studying the effect of foraging effort on nutrient balancing behavior and physiology, and its consequences for performance. We investigated the effect of foraging distance on individual German cockroaches by providing two foods differing in protein-to-carbohydrate ratio at opposite ends of long containers or adjacent to each other in short containers. Each food was nutritionally imbalanced, but the two foods were nutritionally complementary, allowing optimal foraging by selective feeding from both foods. We measured nutrient-specific consumption in fifth instar nymphs and newly eclosed females foraging at the two distances, hypothesizing that individuals foraging over longer distance would select more carbohydrate-biased diets to compensate for the energetic cost of locomotion. We then determined dry mass growth and lipid accumulation in the nymphs as well as mass gain and the length of basal oocytes in the adult females as an estimate of sexual maturation. Nymphs foraging over longer distance accumulated less lipid relative to total dry mass growth, but contrary to our predictions, their protein intake was higher and they accumulated more structural mass. In concordance, adult females foraging over longer distance gained more body mass and matured their oocytes faster. Our results show a positive effect of foraging distance on fitness-related parameters at two life stages, in both cases involving increased consumption of specific nutrients corresponding to requirements at the respective life stage.

KEY WORDS: Carbohydrate, Foraging effort, Juvenile development, Nutrient regulation, Protein, Sexual maturation

INTRODUCTION

In nature, foods are often patchily distributed. Investigations of optimal foraging theory (Pyke, 1984) have largely focused on energy budgets, i.e. caloric intake and expenditure (Stephens and Krebs, 1986), but have concentrated less on whether animals attained a balanced or optimal consumption of specific nutrients. Typically, there are both energetic costs and predation risk associated with high foraging effort, which is often required to locate food sources that may be patchily distributed in the environment (Kennedy and Gray, 1993). Costs associated with foraging can become magnified when the distribution of food becomes increasingly irregular (Kennedy and Gray, 1993), the

habitat increases in complexity (Brown and Kotler, 2004) and when predation threat is present (Lima and Dill, 1990), and all have the potential to influence foraging behavior and physiology, and the resulting performance outcomes.

Polyphagous animals must locate different food sources in order to compose a nutritionally complete diet, which may require foraging over wide distances (Bernays et al., 1994; Lefcheck et al., 2013; Marques et al., 2015). While the consumption of one food alone does not satisfy all nutritional needs, selective consumption of an assortment of foods allows an individual to compose a balanced diet (Simpson and Raubenheimer, 1996; Simpson and Simpson, 1992; Thompson and Redak, 2000), and consequently maximize fitness-determining traits such as growth and reproduction (Jensen et al., 2012, 2013; Lee et al., 2008; Raubenheimer and Jones, 2006; Simpson et al., 2004, 2015). Indeed, a wide range of animals are shown to self-select a balanced diet when given dietary choice (Simpson and Raubenheimer, 2012). However, studies on nutrient self-selection and consequent performance have typically been conducted in small enclosures with complementary foods located adjacent to each other, requiring limited activity and effort to attain a balanced diet. In the wild, foraging effort is required to locate spatially separated food sources, involving energy expenditure as well as learning and memory. Yet, we know little about how foraging distance and environmental complexity affect foraging decisions and nutrient balancing behavior and physiology, and the effect on fitness-related outcomes.

A few studies have examined the effect of distance on food mixing and nutrient balancing in grasshopper nymphs (Behmer et al., 2003; Bernays et al., 1997; van der Zee et al., 2002). Using artificial foods, Behmer et al. (2003) found that migratory locust (*Locusta migratoria*) nymphs regulated their intake to a similar protein versus carbohydrate intake and grew at the same rate regardless of foraging distance. Over time, however, the nymphs tended to spend more time in the area of the arena containing the protein-biased food. Surprisingly, body lipid content tended to be higher in locusts that had foraged in arenas with longer distance between foods (Behmer et al., 2003). In another study, van der Zee et al. (2002) compared consumption of protein- and carbohydrate-biased foods at varying distances in desert locust (*Schistocerca gregaria*) nymphs. Whereas the carbohydrate-biased food was generally preferred, food-shifting frequency declined at longer distance between the complementary foods, and at the longest distance (2 m), nymphs tended to stay longer at the protein-biased food and consumed more from this food (van der Zee et al., 2002). Although these studies showed some effect of distance on nutrient balancing and performance-related outcomes, the effects were subtle and performance-related outcomes were not significant. Furthermore, while herbivores need to balance nutrients from different plants or plant parts, omnivorous foragers typically need to search and explore their environment efficiently to locate foods that are sparse and difficult to find (Clark, 1982). Search effort to attain a

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balanced diet might therefore be more pronounced in omnivorous than in herbivorous animals.

The German cockroach *Blattella germanica* Linnaeus 1767 (Dictyoptera: Blattellidae), is an ideal model omnivore with which to test hypotheses regarding foraging behavior and physiology in structurally complex environments. These highly mobile generalist scavengers are a cosmopolitan pest in human establishments such as homes, restaurants and factories, all of which are structurally complex and contain patchy distributions of nutritional resources (Rust et al., 1995; Schal, 2011; Schal et al., 1984). They possess a highly evolved sensory system that helps them navigate in their environment and can efficiently explore wide areas in search of resources (Durier and Rivault, 2001; Rivault and Cloarec, 1991; Silverman, 1986). Furthermore, they forage in an environment where the availability and nutritional composition of food sources often fluctuate over time and space, which has selected for unique metabolic adaptations to cope with scarce and intermittently available resources (Mullins, 2015). Consequently, German cockroach nymphs are particularly adept at maintaining nutrients and attaining an overall nutritionally balanced diet that maximizes growth despite fluctuations in the availability of nutritionally complementary foods (Jones and Raubenheimer, 2001; Raubenheimer and Jones, 2006).

To investigate the effect of foraging effort on specific nutrient consumption and utilization, and on consequences for fitness-determining traits (growth, lipid accumulation and sexual maturation), we gave newly molted fifth instar German cockroach nymphs and newly eclosed adult females the opportunity to balance their diet from two nutritionally complementary foods placed either adjacent to each other in a small arena with simple physical structure, or at opposite ends of a long arena with a complex physical structure. We hypothesized that greater foraging effort at longer distance would elicit greater consumption of carbohydrate to compensate for energetic expenditure, and furthermore, that longer foraging distance would impose costs on nymphal development and female sexual maturation.

MATERIALS AND METHODS

Experimental animals

The German cockroaches (*B. germanica*) used in our experiments originated from the Orlando Normal strain, which was originally collected in Orlando, Florida, and has been maintained as a standard laboratory strain for more than 60 years (Silverman and Bieman, 1993). The culture was maintained in the laboratory on *ad libitum* water and rodent chow (Purina 5001 Rodent Diet, PMI Nutrition International, St Louis, MO, USA) in transparent plastic containers (46×23×30 cm high). Before experiments started, nymphs were collected from the culture and maintained in a smaller transparent plastic container (18×12×8 cm high) with *ad libitum* rodent chow and water until they molted. The nymphs were checked daily and were set up in the experiment within 24 h of molting to the 5th instar or eclosing as adult females. Rearing and experiments were performed at 25±2°C, 35±5% RH on a 12 h light:12 h dark regime.

Complementary foods and measuring consumption

We produced two synthetic foods varying only in protein-to-carbohydrate (P:C) ratio. The carbohydrate-rich food contained a P:C ratio of 4:56, and the protein-rich food contained a P:C ratio of 47:13. Recipes for the two foods are presented in Table 1. Each food alone constituted a nutritionally imbalanced diet, but in combination, the two foods were complementary and allowed

Table 1. Mass-based ingredient composition of the two complementary foods used in the experiments. The two foods differ only in protein-to-carbohydrate (P:C) ratio

P:C ratio	47:13	4:56
Protein*	47.00	4.11
Casein	23.50	2.05
Peptone	11.75	1.03
Albumin	11.75	1.03
Carbohydrate [‡]	13.00	55.89
Cellulose	26.90	26.90
Agar	4.00	4.00
Vitamins [§]	0.81	0.81

*Includes casein, peptone and albumin; [‡]glucose; [§]Vanderzant vitamin mixture.

individuals to attain a balanced diet where fitness-determining parameters are maximized by self-selecting from the two foods (Jensen et al., 2015; Simpson et al., 2015). The two foods were chosen because the intake target of both nymphal and adult female German cockroaches lies around a P:C ratio of 1:2 (Jensen et al., 2015; Raubenheimer and Jones, 2006), which is approximately equidistant between the P:C ratios of the two experimental foods and allows cockroaches to potentially forage over a broad nutritional range. Foods were freeze dried and presented as biscuit-like cubes (Jones and Raubenheimer, 2001) in inverted plastic vial caps (15 mm diameter, 10 mm high). Before and after feeding, food cubes were dried to constant mass at 50°C for at least 2 days and weighed to the nearest 10 µg. Consumption from each food was calculated as the difference in food dry mass before and after feeding. Protein and carbohydrate intake from each food was then calculated by multiplying the mass consumed with the proportion of each specific nutrient in the food, and total mass of protein and carbohydrate consumed was calculated by summing specific nutrient mass consumed from the two foods.

Manipulating foraging effort

Each arena housed only one newly molted 5th instar nymph or one newly eclosed adult female. All experimental animals were weighed to the nearest 10 µg and allocated at random to one of two foraging arena sizes for seven days, after which cockroaches and the remaining foods were collected. We used short (18.5×13×9.5 cm high) and long (122×10.5×12 cm high) transparent plastic containers to manipulate foraging distance. To further increase environmental complexity within the long containers, we packed each with a series of egg cardboard cartons that exactly matched the width of the container. Thus, there was no specific harborage as insects could shelter under any of the egg cartons. Furthermore, in long foraging arenas it was not possible for an insect to travel in a straight line from one end of the container to the other end without traversing between the egg carton labyrinths. The short arenas in contrast contained just one egg carton section (5×5 cm) to provide harborage while minimizing environmental complexity. We placed the two complementary foods adjacent to each other in the center of the short containers and at the opposite ends in the long containers. The inner walls of all containers were covered with a thin layer of petroleum jelly and mineral oil mixture to prevent climbing. Water was provided in 15.0×2.3 cm glass tubes plugged with cotton. One water tube was provided in the small containers, and one water tube was provided at each end of the long containers plus one in the middle to ensure that water availability did not influence foraging. Water and both complementary foods were provided in amounts exceeding *ad libitum* consumption.

Body measurements

Nymphs ($N=43$ females and 36 males) and adult females ($N=80$) entered the experiment within 24 h of molting into their 5th instar or eclosing to the adult stage. To obtain dry mass and lean dry mass (dry mass after lipid extraction) of nymphs after 7 days in the experiment, each nymph was sexed, weighed (fresh body mass), killed by freezing at -18°C , dried at 50°C for 3 days, weighed (dry body mass), lipid extracted (3 days in 10 ml anhydrous ethyl ether followed by drying at 50°C) and weighed again to obtain lean dry body mass. Individual insect lipid content was calculated by subtracting lean dry mass from dry mass. Seven newly molted 5th instar nymphs were treated in the same manner to obtain the ratio of dry mass to lean dry mass of nymphs entering the experiment. The initial individual dry and lean dry masses of experimental insects were estimated from their initial live mass using linear regressions ($R^2=0.92$ and 0.82 , respectively), based on the group of nymphs that were killed at the start of the experiment. Dry mass growth, lean dry mass growth, and lipid accumulation during the experiment were then calculated by subtracting the estimated initial values from the values measured at the end of the experiment. Mass gain in the adult females was calculated by subtracting the initial body mass from the body mass at the end of the experiment. The females were then dissected and the length of three random basal oocytes was measured with an eyepiece micrometer in a dissecting microscope. The average of the three oocyte lengths was used as the individual measure for analysis. Since all basal oocytes of the German cockroach mature synchronously, there was little variation among the three oocytes within a female.

Statistical analysis

Overall effects of distance and nymph sex on intake of dry mass, protein and carbohydrate were first analyzed using multivariate analysis of covariance (MANCOVA) within nymphs or adult females, using initial body mass as covariate. One adult female from each distance treatment was excluded from consumption analysis because of unusually low consumption that compromised the assumption of normal distribution. To analyze the influence of distance, and of nymph sex, on the specific intake of protein and carbohydrate, the MANCOVAs were followed by one- or two-way analysis of covariance (ANCOVA) with initial body mass as covariate. Nymphal dry mass growth, lean dry mass growth, lipid accumulation and lipid accumulation relative to total dry mass growth were analyzed using similar ANCOVA tests with initial body mass as covariate. Data on proportional lipid accumulation were arcsine transformed before analysis (Zar, 1999). Nymphal dry mass growth was further compared between distances and sexes using a Tukey HSD test. Adult female mass gain and average basal oocyte length were compared using proportional hazard tests with initial body mass as covariate because these data were not normally distributed (Shapiro–Wilk test, $P<0.05$). Utilization of ingested protein for lean dry mass growth in the nymphs was analysed using ANCOVA with lean dry mass growth as dependent variable, distance and sex as factors, and protein intake as covariate. Similarly, utilization of ingested carbohydrate for lipid accumulation and in the nymphs was analysed using ANCOVA with lipid accumulation as dependent variable, distance and sex as factors, and carbohydrate intake as covariate. Adult female utilization of ingested protein and carbohydrate for mass gain and basal oocyte growth were analysed using proportional hazard tests with distance as factor and protein or carbohydrate intake as covariate. Statistical analyses were performed in JMP 12.1.0 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Nymphal feeding and growth

Female nymphs entering the experiment weighed 33.60 ± 0.79 mg (mean \pm s.e.m.), and their initial body masses did not differ significantly between the two distances (t -test: $t_{43}=0.50$, $P=0.48$). Male nymphs weighed 29.39 ± 0.45 mg, and their body masses likewise did not differ significantly between the two treatments (t -test: $t_{36}=3.47$, $P=0.07$). Overall consumption of food and specific nutrients differed significantly both due to distance and sex over the 7 day experiment (Table 2), with higher overall intake of food and of both protein and carbohydrate in nymphs that had foraged over the long distance (Table 3, Fig. 1). Female and male nymphs foraging over the long distance consumed more protein than nymphs of the same sex foraging at the short distance (Table 3, Fig. 1). Both female and male nymphs foraging over the long distance had larger dry mass growth than nymphs of the same sex foraging at the short distance (Table 3, Fig. 2). Similarly, their structural growth measured as lean dry mass growth was greater in individuals foraging over the long distance than in individuals foraging at the short distance within both sexes (Table 3, Fig. 3). In contrast, although lipid accumulation was higher at long than at short distance (Table 3, Fig. 3), both female and male nymphs accumulated less lipid relative to their respective dry and lean dry body mass growth at long distance than at short distance (Table 3, Fig. 3). Distance thus caused different changes in body composition with structural mass constituting a larger proportion of body mass growth while lipid accumulation was relatively smaller in nymphs that foraged over the long distance. Utilization of ingested protein for lean dry mass growth and of ingested carbohydrate for lipid accumulation did not differ significantly between the two distances (Table 4).

Female feeding and sexual maturation

Newly eclosed adult females weighed 58.53 ± 0.75 mg, and initial body masses did not differ significantly between the two distance treatments (t -test: $t_{79}=1.99$, $P=0.16$). Overall differences in consumption of dry mass, protein, and carbohydrate between the two distances were nearly significant (Table 5). While total food intake and protein consumption did not differ between the two distances (Table 6), there was a tendency towards greater carbohydrate consumption (Table 6, Fig. 4) and larger mass gain (Table 6, Fig. 5A) in females foraging over the long distance than in females foraging at short distance. Importantly, females foraging over the long distance matured significantly larger basal oocytes by the end of the experiment than females that had foraged at short distance (Table 6, Fig. 5B). Whereas carbohydrate intake significantly affected oocyte lengths (Table 7), we did not find evidence that carbohydrate was utilized differently for body mass gain or basal oocyte growth between the two distance treatments (Table 7). Ingested protein appeared to be utilized significantly more efficiently for oocyte growth in females that foraged over the longer distance than in females foraging at short distance (Table 7).

Table 2. MANCOVA test, with initial body mass as the covariate, testing for the effects of distance and sex on overall differences in dry mass intake, protein intake, and carbohydrate intake in the last instar nymphs over the 7 day experiment

Factor	d.f.	<i>F</i>	<i>P</i>
Distance	1	36.5856	<0.0001
Sex	1	75.3137	<0.0001
Distance \times sex	1	2.0255	0.1589
Initial body mass	1	5.1762	0.0258

Table 3. ANCOVA tests, with initial body mass as the covariate, testing for the effects of distance and sex on the univariate differences in dry mass intake, protein intake, carbohydrate intake, dry mass growth, lipid accumulation and proportional lipid growth in the last instar nymphs

Dependent variable	Factor	d.f.	t-ratio	P
Dry mass intake	Distance	1	6.05	<0.0001
	Sex	1	8.68	<0.0001
	Distance×sex	1	1.42	0.1589
	Initial body mass	1	2.28	0.0258
Protein intake	Distance	1	6.72	<0.0001
	Sex	1	8.29	<0.0001
	Distance×sex	1	0.67	0.5046
	Initial body mass	1	-0.54	0.5886
Carbohydrate intake	Distance	1	2.10	0.0391
	Sex	1	4.17	<0.0001
	Distance×sex	1	1.27	0.2063
	Initial body mass	1	3.42	0.0010
Dry mass growth	Distance	1	6.35	<0.0001
	Sex	1	8.69	<0.0001
	Distance×sex	1	1.21	0.2312
	Initial body mass	1	-3.15	0.0024
Lean dry mass growth	Distance	1	6.69	<0.0001
	Sex	1	9.06	<0.0001
	Distance×sex	1	1.17	0.2440
	Initial body mass	1	-4.71	<0.0001
Lipid accumulation	Distance	1	2.66	0.0095
	Sex	1	3.87	0.0002
	Distance×sex	1	0.71	0.4771
	Initial body mass	1	1.67	0.0984
% Lipid growth	Distance	1	-3.90	0.0002
	Sex	1	-4.87	<0.0001
	Distance×sex	1	0.42	0.6788
	Initial body mass	1	5.07	<0.0001

Since protein intake did not differ between the two distances, different outcomes relating to protein intake makes sense owing to the larger carbohydrate consumption and larger basal oocyte growth by females foraging over the long distance, in combination with the

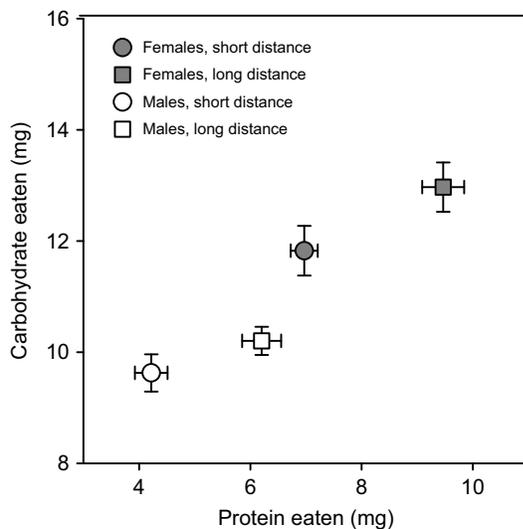


Fig. 1. Protein and carbohydrate intake in female and male *Blattella germanica* nymphs foraging between nutritionally complementary foods over either long or short distance over 7 days. Nymphs were set up individually in arenas with long or short distance between complementary foods, within 24 h of molting to the 5th instar. There were 16 replicates of female and 23 of male nymphs in the long distance treatment and 27 female and 13 male nymphs in the short distance treatment. Values are means±s.e.m.

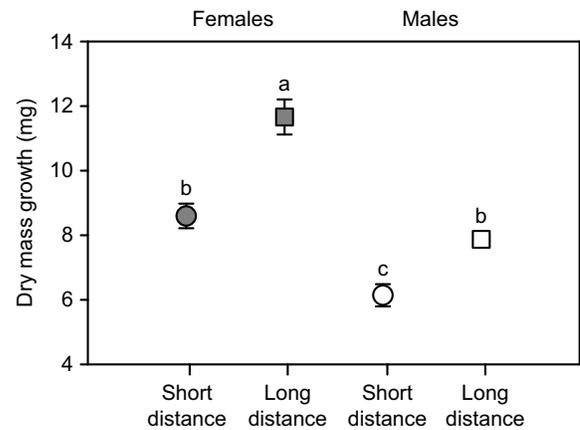


Fig. 2. Dry mass growth in *Blattella germanica* nymphs over the 7 day feeding period. Different letters indicate significant differences (Tukey HSD test, $P<0.05$). Nymphs were set up individually in arenas with long (16 female and 23 male nymphs) or short (27 female and 13 male nymphs) distance between complementary foods, within 24 h of molting to the 5th instar. Values are means±s.e.m.

direct positive effect of carbohydrate intake on basal oocyte length (Table 7). However, we do not know how much of the ingested protein was allocated to the oocytes.

DISCUSSION

Animals must consume foods in optimal ratios and amounts in order to optimally balance their intake of specific nutrients and maximize performance (Jensen et al., 2012; Lee et al., 2008; Rapport, 1980; Simpson and Raubenheimer, 2012; Simpson et al., 2004, 2015). When foraging for a nutritionally complete diet in a complex environment, a search effort involving locomotory activity is required to compose an optimal diet from complementary resources that are patchily distributed and may be difficult to find. Using German cockroaches as a model omnivore, we found evidence that

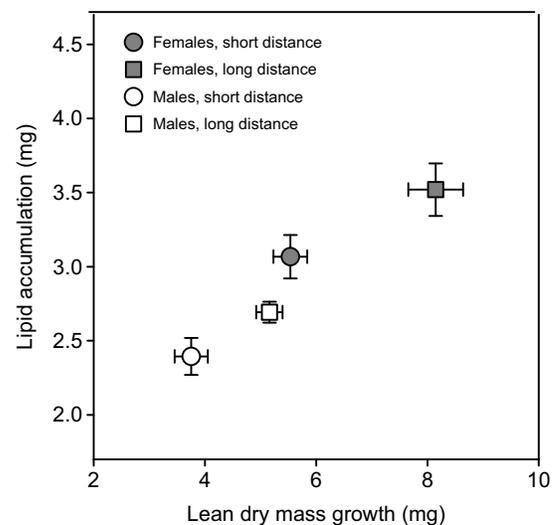


Fig. 3. Lean dry mass growth and lipid accumulation during the 7 day feeding period in the female and male *Blattella germanica* nymphs foraging between nutritionally complementary foods over either long or short distance. Nymphs were set up individually in arenas with long (16 female and 23 male nymphs) or short (27 female and 13 male nymphs) distance between complementary foods, within 24 h of molting to the 5th instar. Values are means±s.e.m.

Table 4. ANCOVA tests of the utilization of ingested protein and carbohydrate for lean dry mass growth and lipid accumulation in the last instar nymphs

Dependent variable	Constituent	Factor	d.f.	t-ratio	P
Lean dry mass growth	Protein	Distance	1	1.45	0.1514
		Sex	1	0.89	0.3745
		Distance×sex	1	1.61	0.1115
		Protein intake	1	7.37	<0.0001
Lipid accumulation	Carbohydrate	Distance	1	1.67	0.0996
		Sex	1	0.35	0.7259
		Distance×sex	1	-0.19	0.8525
		Carbohydrate intake	1	12.01	<0.0001

foraging for complementary nutrients over a distance enhanced the ingestion of specific nutrients and lead to higher performance in fitness-related traits, including nymphal growth and female sexual maturation.

We hypothesized that greater foraging effort would elicit greater consumption of carbohydrate to compensate for energetic expenditure. Contrary to this hypothesis, we found that nymphs of both sexes had higher protein consumption rather than higher carbohydrate consumption when foraging over the longer distance compared with nymphs foraging at short distance. Although higher energetic requirements and therefore a requirement for more carbohydrate would be expected when foraging over a longer distance, our finding is in concordance with previous studies on locust nymphs which similarly indicated a higher preference for protein at longer distance between complementary foods (Behmer et al., 2003; van der Zee et al., 2002). As observed in the previous experiments, the higher consumption of protein from two complementary food sources at longer distance may be due to higher fidelity to a protein-rich food when the distance between food sources is longer. From an ecological perspective, this makes sense because of the typical scarcity of protein relative to carbohydrate available to omnivores and herbivores within most environments (Mattson, 1980; White, 1993), most likely including that of the German cockroach (Bell et al., 2007; Kells et al., 1999). If protein-rich food sources are required, for example for growth, but are typically rare in the environments where cockroaches have evolved, they are therefore likely to be perceived as more valuable than carbohydrate-rich sources once located, and larger amounts should be ingested before searching for complementary food. The gluconeogenic pathway is present in the American cockroach (*Periplaneta americana*) but functions only at a low rate (Sevala and Steele, 1989). German cockroaches do not appear to be capable of efficiently utilizing protein as an energy source (Jensen et al., 2015; Raubenheimer and Jones, 2006), which is probably due in part to a low capacity for gluconeogenesis and in part to a build-up of nitrogenous waste products due to limited nitrogen excretion (Cochran, 1985). Whereas the previous studies on locust nymphs did not find effects of foraging distance on performance parameters (Behmer et al., 2003; van der Zee et al., 2002), we found larger

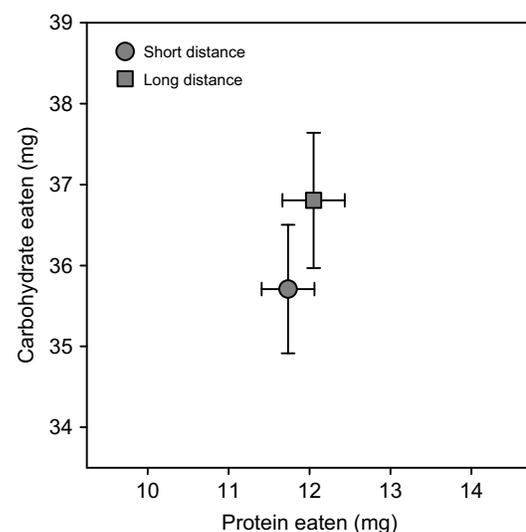
Table 5. MANCOVA test, with initial body mass as the covariate, testing for the effect of distance on overall differences in dry mass intake, protein intake, and carbohydrate intake in the adult females over the 7 day experiment

Factor	d.f.	F	P
Distance	1	3.5096	0.0650
Initial body mass	1	15.5516	0.0002

Table 6. ANCOVA and proportional hazard tests, with initial body mass as the covariate, testing for the effects of distance on the univariate differences in dry mass intake, protein intake, and carbohydrate intake, mass gain and basal oocyte length in the adult females

Dependent variable	Factor	d.f.	t-ratio	χ^2	P
Dry mass intake	Distance	1	1.87	–	0.0650
	Initial body mass	1	3.94	–	0.0002
Protein intake	Distance	1	0.48	–	0.6311
	Initial body mass	1	0.30	–	0.7620
Carbohydrate intake	Distance	1	1.99	–	0.0501
	Initial body mass	1	4.59	–	<0.0001
Mass gain	Distance	1	–	2.22	0.1361
	Initial body mass	1	–	2.84	0.0918
Basal oocyte length	Distance	1	–	5.85	0.0156
	Initial body mass	1	–	5.18	0.0228

growth in both male and female cockroach nymphs that foraged over the longer distance compared with those foraging at short distance (Table 3, Fig. 2). This shows that there was a performance benefit associated with active foraging in German cockroaches, which corresponds well with the higher consumption in animals that foraged over longer distance. In addition, we found faster maturation of basal oocytes in virgin females that foraged over the longer distance (Table 6, Fig. 5B), which means that there would be a shorter time until females are ready to mate and initiate reproduction. The tendency of adult females to consume higher levels of carbohydrate rather than protein when foraging over the longer distance probably reflects the fact that sexual maturation in female cockroaches first of all requires energy to produce lipids for the maturing oocytes (Ziegler and Van Antwerpen, 2006). Protein-rich foods are expected to increase in value as yolk is provisioned to oocytes and vitellogenesis rates increase, but lipid accumulation appears to be prioritized at this relatively early stage of the gonadotropic cycle. Thus, both nymphs and adult female cockroaches foraging at the longer distance appeared to ingest more of the specific nutrients they required in order to maximize performance at their respective life stage. Such a demonstration of

**Fig. 4. Protein and carbohydrate intake in adult *Blattella germanica* females foraging between nutritionally complementary foods over either long or short distance over 7 days.** The female cockroaches were set up individually in foraging arenas within 24 h of eclosion to the adult stage. Values are means±s.e.m. of 39 replicates of adult females foraging at each distance.

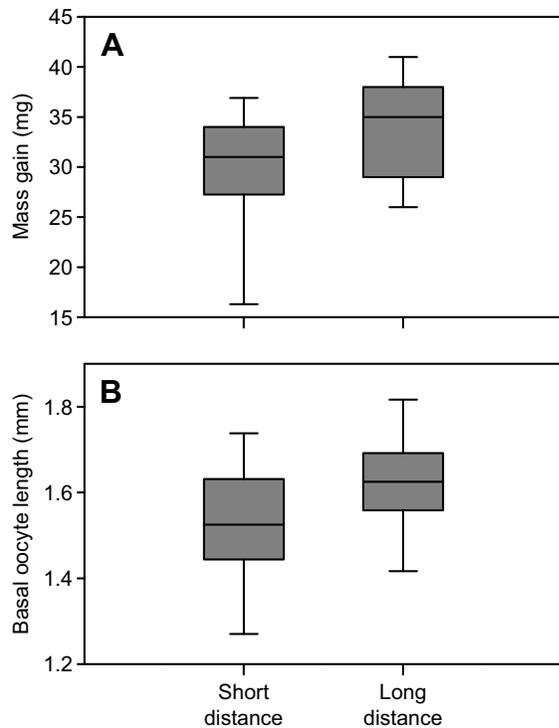


Fig. 5. Performance-related outcomes in adult *Blattella germanica* females after a 7-day feeding period. Each foraging distance included 40 replicates of adult females set up individually within 24 h of eclosion to the adult stage. (A) Mass gain. (B) Average basal oocyte length. Values are medians and 10th, 25th, 75th and 90th percentiles.

increased performance due to higher nutrient-specific consumption when foraging over a longer distance has never been reported before.

Increased protein intake and fidelity to a protein-rich food at longer foraging distance might also be associated with the greater danger that follows increased searching activity because of the higher risk of encountering a predator. Higher protein intake is known to increase growth rate in insects and other invertebrates (Behmer, 2009; Jensen et al., 2011, 2013), and individuals with a higher growth rate can more rapidly escape predation from similar-sized predators as they become too large to handle (Ludwig and Rowe, 1990; Price et al., 1980). At a higher growth rate, individuals are also more likely to reach the adult stage and reproduce before being killed by a predator. Higher protein intake and growth rate at the longer foraging distance could therefore be a means for nymphs to escape predation from smaller-sized predators and start

Table 7. ANCOVA tests of the utilization of ingested protein and carbohydrate for mass gain and basal oocyte length in the adult females

Dependent variable	Constituent	Factor	d.f.	<i>t</i> -ratio	<i>P</i>
Mass gain	Protein	Distance	1	3.4491	0.0633
		Protein intake	1	7.9094	0.0049
	Carbohydrate	Distance	1	2.6415	0.1041
		Carbohydrate intake	1	1.3401	0.2470
Basal oocyte length	Protein	Distance	1	4.1489	0.0417
		Protein intake	1	5.6306	0.0176
	Carbohydrate	Distance	1	2.2341	0.1357
		Carbohydrate intake	1	9.9677	0.0017

reproducing sooner. In wood cricket (*Nemobius sylvestris*) nymphs, feeding as well as weight gain appeared to be stimulated by spider cues (Bucher et al., 2014). Furthermore, grasshopper (*Melanoplus femurrubrum*) nymphs that were exposed to a spider predator consumed more food than unexposed nymphs, but the difference was due to a higher consumption of carbohydrate and not protein in predator-exposed nymphs (Hawlena and Schmitz, 2010). Clearly, more studies are required to disentangle the effects of foraging distance and risk of predation on specific nutrient consumption.

In the study by Behmer et al. (2003), locust nymphs that foraged in large arenas with longer distances between complementary foods tended to contain more lipid than nymphs foraging in small arenas with shorter distances between foods. We similarly found larger lipid stores in German cockroach nymphs that had foraged over longer distance (Table 3, Fig. 3). However, lipid accumulation relative to dry and lean dry mass growth was smaller in nymphs foraging over the longer distance than in nymphs foraging at short distance (Table 3, Fig. 3), which corresponds well with the higher increase in protein intake relative to carbohydrate intake in nymphs foraging over the long distance. Furthermore, a larger proportion of the ingested energy might have been respired in nymphs foraging at long distance owing to the energetic costs associated with body growth and with active searching of their environment, although this was not significant (Table 4). Still, the long distance in our experiment is most likely rather short relative to distances that cockroaches travel to obtain complete diets in the field, and German cockroaches are known to be capable of foraging efficiently over a much wider area (Rivault and Cloarec, 1991) than in the present experiment.

We did not find evidence clearly indicating different utilization of ingested nutrients by cockroaches foraging over long compared with short distance (Tables 4 and 7). When there is a chance to balance nutrients by regulating ingestion at relatively short distances as in the present experiment, the cost of traveling between complementary foods to balance nutrients is presumably smaller than the cost of regulating nutrients after ingestion. The positive correlations between nutrient-specific consumption and performance outcomes further indicate that ingested nutrients were utilized at similar efficiency regardless of foraging distance. Different utilization of specific nutrients after ingestion has been shown in a number of invertebrates when restricted to nutritionally imbalanced diets over longer periods (Jensen et al., 2011, 2013; Lee et al., 2002, 2003; Simpson et al., 2002), and involves reduced utilization efficiency of the abundant nutrient to facilitate larger overall consumption and therefore larger ingestion of the deficient nutrient. At longer distances between individually imbalanced food sources than in the present experiment, different utilization of ingested nutrients would be likely to occur if individuals stay increasingly longer at located foods as the distance between foods increases, and they would therefore ingest more nutritionally imbalanced food before searching for a complementary food source (Behmer et al., 2003; Bernays et al., 1997).

The present study provides a good example of how environmental conditions may interact with physiological responses and affect fitness-determining life-history characteristics, as reviewed by Lailvaux and Husak (2014). An interesting question that arises from our study is whether increased activity associated with searching the environment has a direct reinforcing effect on body growth by influencing hormone levels. As higher foraging activity increases predation risk (Brown and Kotler, 2004), a longer distance

between foods could theoretically induce hormonal signals that stimulate faster growth and earlier reproduction (McPeck, 2004). In humans, it is well established that exercise induces increased levels of growth hormone (Godfrey et al., 2003). Larger lean body mass and oocyte growth at the longer distance indicates that cockroaches foraging over the longer distance probably had higher levels of juvenile hormone or another growth stimulating hormone. This could be caused by the higher levels of consumption, or possibly by higher activity associated with foraging. Accelerated growth and sexual maturation caused by distance between complementary resources has never been demonstrated before and warrants further investigation. While additional studies investigating the mechanistic underpinnings of these phenomena are still needed, this study represents a first step in describing the effect of foraging distance on life-history and fitness-determining traits in a mobile and explorative omnivore.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

A.E.K and K.J. conducted the experiment; K.J. and A.E.K. analyzed the data; K.J., A.E.K., J.S. and C.S. wrote the manuscript.

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