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Diet specialization in an extreme omnivore: nutritional regulation in glucose-averse German cockroaches

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Abstract

Organisms have diverse adaptations for balancing dietary nutrients, but often face trade-offs between ingesting nutrients and toxins in food. While extremely omnivorous cockroaches would seem excluded from such dietary trade-offs, German cockroaches (*Blattella germanica*) in multiple populations have rapidly evolved a unique dietary specialization – an aversion to glucose, the phagostimulant in toxic baits used for pest control. We used factorial feeding experiments within the geometric framework to test whether glucose-averse (GA) cockroaches with limited access to this critical metabolic fuel have compensatory behavioural and physiological strategies for meeting nutritional requirements. GA cockroaches had severely constrained intake, fat and N mass, and performance on glucose-based diets relative to wild-type (WT) cockroaches and did not appear to exhibit digestive strategies for retaining undereaten nutrients. However, a GA × WT 'hybrid' had lower glucose aversion than GA and greater access to macronutrients within glucose-based diets – while still having lower intake and survival than WT. Given these intermediate foraging constraints, hybrids may be a reservoir for this maladaptive trait in the absence of positive selection and may account for the rapid evolution of this trait following bait application.

Introduction

Organisms have diverse adaptations for optimally blending dietary nutrients required for growth, maintenance and reproduction (Simpson & Raubenheimer, 2012). However, toxic or unpalatable compounds in food often complicate nutrient-balancing strategies (Agrawal & Klein, 2000; Simpson & Raubenheimer, 2001; Behmer *et al.*, 2002), and intake often reflects trade-offs between benefits of ingesting nutrients and costs of processing toxins (Freeland & Janzen, 1974; Rapport, 1980). However, while deterrent compounds can be most effective in nutritionally poor food (Raubenheimer, 1992; Behmer *et al.*, 2002), the shapes of nutrient–toxin trade-offs likely depend on consumer diet breadth (Lee *et al.*, 2006). We use a remarkable adaptive response to dietary toxins in a cockroach to explore links between the evolution of dietary specialization and compensatory strategies for nutrient regulation.

Most cockroaches are extremely opportunistic omnivores that can mix diverse foods to avoid dietary trade-offs (Schal *et al.*, 1984; Jones & Raubenheimer, 2001; Raubenheimer & Jones, 2006). However, the German cockroach (*Blattella germanica*, Linnaeus) has rapidly (within 5 years) evolved a unique dietary specialization in response to toxic baits used in pest control. Rather than evolving resistance to the toxicant, individuals in multiple populations have become averse to the bait phagostimulant glucose (Silverman & Bieman, 1993; Silverman & Ross, 1994), while readily consuming other sugars, including fructose (Silverman & Bieman, 1993; Wada-Katsumata *et al.*, 2011). Glucose aversion (GA) is mediated by heritable (Silverman & Bieman, 1993; Ross & Silverman, 1995) changes in mouthpart gustatory neurons that misinterpret glucose as a bitter compound, even though glucose is not toxic

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to GA cockroaches (Wada-Katsumata *et al.*, 2013). GA cockroaches thus provide an exceptional opportunity to study the evolution of nutritional trade-offs, and pre- and post-ingestive solutions to nutrient procurement with limited access to glucose – a critical metabolic fuel.

GA nymphs suffer reduced growth and survival rather than consume glucose (Silverman, 1995). And yet, rapid parallel evolution of this costly trait in multiple populations in response to glucose-containing toxic baits (Silverman & Ross, 1994; Wang *et al.*, 2004) suggests the GA trait can be present at low frequency, even without this selective force. Here, we use a factorial feeding experiment within the geometric framework (Simpson & Raubenheimer, 2012) to test whether glucose aversion is associated with compensatory strategies for nutritional regulation. We first let cockroaches 'reveal' their macronutrient intake targets (IT) through compensatory feeding between two diets with known protein and carbohydrate content. We then confine cockroaches to diets with suboptimal protein:carbohydrate ratios to assess rules of compromise (RoC) – assumed to be adaptive strategies for prioritizing one macronutrient at the cost of overeating or undereating another.

The evolution of carbohydrate requirements

One solution to glucose aversion would be to evolve lower carbohydrate requirements. This would be supported if GA cockroaches have lower carbohydrate ITs relative to non-GA (wild type; WT) strains – even when fed diets containing palatable sugars. GA cockroaches may have also evolved digestive strategies for balancing nutrients even when foraging away from their ITs (Zanotto *et al.*, 1993; Trier & Mattson, 2003; Kay *et al.*, 2012). *Blattella germanica* is particularly endowed with such strategies, storing excess carbohydrates in the fat body and excreting excess proteins as uric acid or volatile ammonium, while also storing nitrogen in specialized fat body cells containing bacterial endosymbionts (Cochran, 1985; Sabree *et al.*, 2009) and specialized uric acid storage glands (Mullins & Keil, 1980). We thus test whether GA cockroaches are (i) especially efficient at retaining ingested carbohydrates, while excreting nutrients consumed in excess, or (ii) are especially adept at using alternative nutrients to support development. We use factorial feeding experiments to test these pre- and post-ingestive hypotheses, manipulating dietary glucose and macronutrient composition and measuring the performance consequences (e.g. growth and survival) of feeding decisions.

Nutritional regulation in a transitional hybrid

A single incompletely dominant major gene appears to control glucose aversion, and hybrid sensitivity to glucose is intermediate between parental WT and GA strains (Silverman & Bieman, 1993; Ross & Silverman, 1995; Silverman, 1995). If compensatory feeding behaviours in GA x WT hybrids are less restricted by dietary glucose than in GA homozygotes, we posit that hybrids can maintain this trait in populations not exposed to toxic baits. We include GA × WT hybrids in the experiments detailed above and test whether they have distinct nutrient regulation strategies.

Materials and methods

Experimental setup

We performed feeding experiments on two strains of 17-day-old (16.6 ± 0.1 days, ± SEM, $n = 320$) *Blattella germanica* nymphs – wild type (WT; Orlando Normal) and glucose averse (GA; T164), as well as a reciprocally crossed WT × GA 'hybrid'. Glucose aversion is an autosomal trait (Silverman & Bieman, 1993), and we analysed reciprocal crosses as a single 'hybrid strain' because they generally had statistically indistinguishable responses to experimental manipulations (i.e. nonsignificant strain main effects, Table S1). Cockroaches were provided water and food pellets [Purina No. 5001 Rodent Diet, PMI Nutrition International, St. Louis, MO, USA; composition: 23.9% protein, 5% fat, 48.7% nitrogen-free extract (31.9% starch, 0.22% glucose, 0.30% fructose, 3.70% sucrose, 2.01% lactose); this diet contains glucose in quantities too small to induce aversive feeding behaviour in cockroaches] from hatch until entrance into feeding experiments, which were performed in a climate-controlled laboratory at 12 : 12 L : D photoperiod, 26 °C, and 65% RH. All experiments were performed on groups of 10 unsexed nymphs of a given strain placed in 237-mL jars lined with vaseline/mineral oil and covered with paper towel squares. For GA and WT nymphs, we used 10 jars per treatment combination and we used 12 jars per treatment combination for hybrid nymphs ($n = 6$ per reciprocal cross). After a 24-h acclimation period, nymphs were provided *ca*. 500 mg preweighed cube of freeze-dried diet(s). All subjects received *ad lib* water, provided in cotton-plugged microcentrifuge tubes replaced every third day. As described below, we designed two-way (choice experiment: strain, sugar) and three-way (no-choice experiment: strain, diet, sugar) factorial experiments.

Dietary manipulations

We manipulated dietary protein and carbohydrates using agar-based diets modified from Dussutour & Simpson (2008), with protein : carbohydrate (p : c) ratios of 1 : 3 (high carbohydrate; pC), 1 : 1 (even; pc) or 3 : 1 (high protein; Pc). Protein in these diets came from dried whole egg powder, calcium caseinate and whey protein. Vanderzant's vitamin mixture provided micronutrients. The digestible carbohydrate used in the diets was either glucose or fructose. Glucose is accepted by WT and rejected by the T164 GA strain, whereas fructose is accepted by both strains (Silverman & Bieman, 1993; Wada-Katsumata *et al.*, 2011). These p : c ratios span the optimal range accepted by *B. germanica* in prior experiments (Raubenheimer & Jones, 2006). For details, see Appendix S1.

Diet choice experiment

We assessed intake targets (IT) by providing nymphs with two diets (pC and Pc) for 14 days and then calculating per-nymph intake (summed intake for both diets/number of surviving nymphs). Diets were dried for 24 h at 60 °C and weighed to the nearest 1 mg. Initial dry diet mass was estimated with dry : wet ratios from control diets (four per diet and sugar type) placed in empty jars for 14 days. Macronutrient intake was calculated from dietary ratio of protein:carbohydrate. Survival across 64 jars was high (9.5 ± 0.1 SEM of 10 nymphs surviving per jar).

No-choice diet experiment

We assessed rules of compromise (RoC) by modifying the choice set-up to confine nymphs to a Pc, pc or pC diet. After 14 days, we harvested cockroaches, their faeces and the remaining diet. We calculated per-nymph intake (as in the choice experiment), faeces output (faeces dry mass/number of survivors) and growth (nymph final dry mass/number of survivors). Survival across 192 jars was high (9.2 ± 0.1 SEM of 10 nymphs surviving per jar). All cockroaches and their faeces were freeze-dried at −40 °C for 4 days following the experiment and then frozen at −20 °C. No insects reached the adult stage by day 14 of the experiment. We randomly selected two freeze-dried nymphs from each experimental jar for nutritional analyses. In the first individual, we estimated %fat mass using Soxhlet extraction as in Smith & Tschinkel (2009). We analysed the second nymph (as well as the associated faeces) for elemental nitrogen (N mass, a proxy for protein content) using a model 2400 CHN elemental analyser (Perkin Elmer Corp., Waltham, MA, USA). For details about these analyses, see Appendix S1.

Statistics

For intake analyses, we used univariate mixed model analyses (SAS proc GLIMMIX, V9.3, Cary, NC, USA) with initial cockroach mass as a covariate. In the choice experiment, we tested for intake (total, protein, carbohydrate) differences between 'strains' (WT, GA, hybrid) and sugars (fructose- vs. glucose-based diet), and in the no-choice diet experiment, we added diet (pC, pc, Pc) as a class variable. The covariate was included because hybrid nymphs (9.15 ± 0.14 mg) had significantly higher initial body wet mass than GA (7.08 ± 0.19 mg) and WT (7.45 ± 0.14 mg) nymphs at the start of the experiment (GLM: strain, $F_{2,252} = 51.70$, $P = 0.0001$).

We next used mixed models to analyse the effects of strain, sugar and intake on nymph condition and performance (body fat mass, body N mass, faeces N mass, total faeces mass, growth, survival). We first calculated residuals stratified by diet for each variable to remove the effects of initial body mass from each model, using total intake residuals to calculate protein and carbohydrate intake based on dietary p : c ratios. We used carbohydrate intake residuals as a covariate in the analysis of nymph fat mass, protein intake residuals for analyses of N mass in nymph bodies and faeces, and total intake for analyses of faeces mass, growth and survival. We explored all significant interactions with Tukey's tests for simple effects (slice option). In Fig. 1, the protein and carbohydrate intake data for the no-choice experiment are provided with standard errors coincident with intake rails because nymphs were confined to single diets that could not deviate from defined p : c ratios (Warbrick-Smith *et al.*, 2009). When testing for treatment effects on nymph survival in the no-choice diet experiment, we used raw mortality data because initial body mass was not a significant covariate.

Results

Diet choice experiment

When fed fructose-based diets, GA nymphs had a lower carbohydrate intake target (IT) than WT nymphs, but hybrid nymphs had a higher carbohydrate IT than WT ($F_{2,57} = 49.44$, $P = 0.0001$; Table 1; Fig. 1a). Protein ITs, in contrast, did not differ across strains ($F_{2,57} = 3.28$, $P = 0.357$; Table 1; Fig. 1a). As a result, GA nymphs foraged for higher protein:carbohydrate (1 : 1) than WT (3 : 4), and hybrids (2 : 3) foraged for a lower p : c than WT, but with higher total intake ($F_{2,57} = 14.89$, $P = 0.0001$; Table 1; Fig. 1a).

On glucose-based diets, ITs shifted towards protein for both GA (5 : 4) and hybrid (8 : 5) nymphs. GA and hybrid nymphs had similar carbohydrate ITs on glucose-based diets (Tukey, $P = 0.490$; Fig. 1b), and these were lower than their respective carbohydrate ITs on fructose-based diets (Tukey, $P = 0.0001$; Table 1, Fig. 1a). However, while GA nymphs had a lower protein IT on glucose-based than on fructose-based diet (Tukey, $P = 0.0001$), the protein IT for hybrids remained unchanged (Tukey, $P = 0.992$; Fig. 1). Hybrid nymphs thus had greater access to dietary protein than GA nymphs on food containing glucose.

No-choice diet experiment

Intake

When forced on nutritionally imbalanced diets containing fructose, nymphs of all strains similarly defended

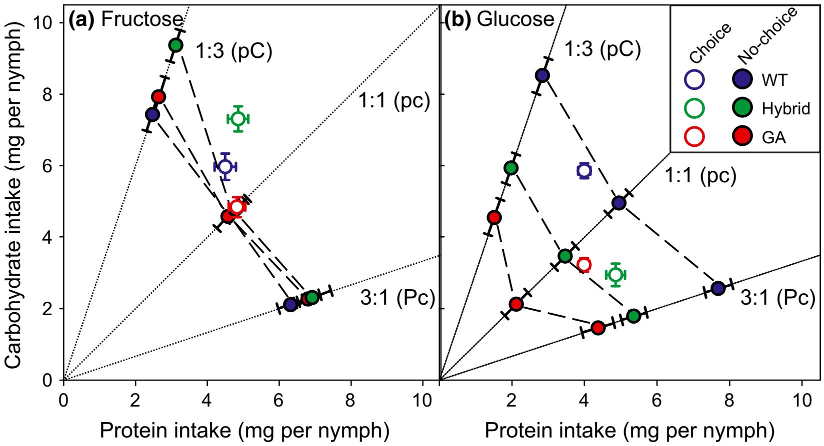

Fig. 1 Macronutrient intake across cockroach strains [wild type (WT), hybrid, glucose averse (GA)], diets (1 : 3, 1 : 1, 3 : 1 protein: carbohydrate) and sugars (fructose, glucose) over 14 days of development. Per-nymph macronutrient intake on diets with either (a) fructose or (b) glucose measured when nymphs were either provided a choice between two suboptimal diets (hollow circles) or were forced to consume a single diet (filled circles). Results of the choice experiment reflect protein and carbohydrate 'intake targets', and results of the no-choice experiment (connected by dashed lines for each strain) represent 'rules of compromise', which are decisions to overeat the surplus macronutrient or undereat the limiting macronutrient. Radial lines extending from the origin indicate nutritional rails corresponding to each diet's protein:carbohydrate ratio. All data points are LS means (±SEM).

protein ITs more closely than carbohydrate ITs. On Pc (3 : 1) fructose-based diets, all strains overate ca. 2 mg of protein, while incurring carbohydrate deficits ranging from 2.6 mg (GA) to 5.0 mg (hybrid) (Fig. 1a). On pC (1 : 3) fructose-based diets, all strains underate ca. 2 mg of protein, while overeating carbohydrates by 1.5 mg (WT) to 3.1 mg (GA) (Fig. 1a).

Strain intake differences emerged on glucose-based diets, with GA nymphs exhibiting a concave intake array, with low intake relative to other strains ($F_{2,163} = 152.12$, $P = 0.0001$; Table 1, Fig. 1b), and particularly low total intake, protein intake and carbohydrate intake on the pc diet ($F_{4,163} = 3.95$, $P = 0.004$; Table 1), although their preferred macronutrient IT was close to this 1 : 1 nutritional rail (Fig. 1b). Hybrid nymphs, in contrast, had a linear intake array on glucose-based diets similar to WT nymphs. However, while hybrid nymphs ate substantially more carbohydrates than GA nymphs on pC and pc diets to more closely approach their protein IT, hybrids still consumed less diet than WT across all glucose-based diets (Tukey, $P = 0.0001$; Fig. 1b). Below, we outline the resulting performance costs.

Post-ingestive responses

Strains had statistically similar, positive relationships between carbohydrate intake and fat mass ($F_{2,160} = 0.44$, $P = 0.642$; Fig. 2) and between protein intake and N body mass ($F_{2,167} = 0.37$, $P = 0.694$; Fig. 3a,b), but these measures of body composition varied across glucose-based diets where GA nymphs had restricted intake relative to hybrids (moderate aversion) and WT nymphs (no aversion). Whereas strains had similar fat mass (Fig. 2a) and N body mass (Fig. 3a) when fed fructose-based diets (Tukey, $P > 0.24$), GA nymphs fed glucose-based diets had lower carbohydrate intake and lower fat mass ($F_{2,160} = 3.96$, $P = 0.021$; Table 1) than WT (Tukey, $P = 0.005$) (Fig. 2b), and lower protein intake and lower N mass ($F_{2,167} = 49.44$, $P = 0.0001$; Table 1) than WT (Tukey, $P = 0.03$) (Fig. 3b). Hybrid nymphs had lower N body mass than WT on glucose-based diets (Tukey, $P = 0.05$), but not lower fat mass ($P = 0.08$).

Strains exhibited similar positive relationships between protein intake and N excretion ($F_{2,167} = 1.02$, $P = 0.364$; Fig. 3c,d) and between total intake and total excretion ($F_{2,167} = 1.67$, $P = 0.257$; Fig. 4a,b). However, although GA and hybrid nymphs had higher N faeces mass (Fig. 3c) and total faeces mass (Fig. 4a) than WT nymphs on fructose-based diets, they had lower N excretion associated with lower protein intake ($F_{2,167} = 8.36$, $P = 0.0003$; Fig. 3d) and lower total excretion associated with lower dietary intake ($F_{2,167} = 7.89$, $P = 0.0005$; Fig. 4b) when fed glucose-based diets (Table 1). This combination of intake constraints and lack of compensatory post-ingestion processing resulted in severe performance costs in GA nymphs and moderate performance costs for hybrid nymphs.

Performance

Nymph growth ($F_{1,167} = 0.67$, $P = 0.512$, Fig. 4c,d) and survival ($F_{1,168} = 0.44$, $P = 0.644$, Fig. 4e,f) were similarly positively related to dietary intake across strains. However, while strain growth (Tukey, $P > 0.30$) and survival (Tukey, $P > 0.58$) were statistically invariant

Table 1 Univariate mixed model analyses testing for differences in the dependent variables (DV) dietary intake (total, protein, carbohydrate), nutrient assimilation (fat mass, N mass), nutrient excretion (N mass, faeces mass) and performance (growth, survival) during cockroach development. The treatments in the three-way factorial design were strain (wild type, hybrid, glucose averse), diet (1 : 3, 1 : 1, 3 : 1 p : c) and sugar (fructose, glucose). Diet was not a factor in the choice experiment analyses because it included a single 1 : 3 and 3 : 1 diet combination, and intake was used as a covariate instead of diet in the analyses of nymph assimilation, excretion and performance.

Experiment	DV	Treatment	d.f.	*F*	*P*
Choice	Total intake	Strain	2	19.95	0.0001
		Sugar	1	76.80	0.0001
		Strain × sugar	2	14.89	0.0001
		Initial mass	1	13.74	0.0005
		Error	57		
	Protein intake	Strain	2	1.05	0.357
		Sugar	1	8.86	0.004
		Strain × sugar	2	3.28	0.045
		Initial mass	1	12.89	0.0007
		Error	57		
	Carbohydrate intake	Strain	2	53.54	0.0001
		Sugar	1	123.46	0.0001
		Strain × sugar	2	49.44	0.0001
		Initial mass	1	6.54	0.013
		Error	57		
No-choice	Total intake	Strain	2	101.54	0.0001
		Diet	2	29.27	0.0001
		Sugar	1	213.32	0.0001
		Strain × diet	4	3.36	0.011
		Strain × sugar	2	152.12	0.0001
		Diet × sugar	2	8.72	0.0003
		Strain × diet × sugar	4	3.95	0.004
		Initial mass	1	120.64	0.0001
		Error	163		
	Protein intake	Strain	2	61.95	0.0001
		Diet	2	448.87	0.0001
		Sugar	1	114.93	0.0001
		Strain × diet	4	7.24	0.0001
		Strain × sugar	2	102.78	0.0001
		Diet × sugar	2	6.23	0.003
		Strain × diet × sugar	4	5.64	0.0003
		Initial mass	1	135.34	0.0001
		Error	163		
	Carbohydrate intake	Strain	2	123.01	0.0001
		Diet	2	1196.19	0.0001
		Sugar	1	246.54	0.0001
		Strain × diet	4	23.98	0.0001
		Strain × sugar	2	142.22	0.0001
		Diet × sugar	2	33.14	0.0001
		Strain × diet × sugar	4	24.84	0.0001
		Initial mass	1	54.22	0.0001
		Error	163		
	Body fat mass	Strain	2	3.96	0.021
		Sugar	1	1.54	0.216
		Strain × sugar	2	1.79	0.170
		Carbohydrate intake	1	16.50	0.0001
		Carb intake × strain	2	0.44	0.642
		Carb intake × sugar	1	0.31	0.580
		Strain × sugar × carb	2	1.11	0.333
		Error	160		
	Body N mass	Strain	2	0.80	0.453
		Sugar	1	3.25	0.073

Table 1 (*Continued*)

Experiment	DV	Treatment	d.f.	*F*	*P*
		Strain × sugar	2	3.75	0.026
		Protein intake	1	56.68	0.0001
		Protein intake × strain	2	0.37	0.694
		Protein intake × sugar	1	0.03	0.874
		Strain × sugar × prot	2	3.03	0.051
		Error	167		
	Faeces N mass	Strain	2	0.80	0.452
		Sugar	1	1.34	0.249
		Strain × sugar	2	8.36	0.0003
		Protein intake	1	24.90	0.0001
		Prot in × strain	2	1.02	0.364
		Protein in × sugar	1	0.01	0.913
		Strain × sugar × prot	2	1.54	0.218
		Error	167		
	Faeces mass	Strain	2	2.65	0.074
		Sugar	1	4.71	0.031
		Strain × sugar	2	7.89	0.0005
		Total intake	1	17.88	0.0001
		Total in × strain	2	1.37	0.257
		Total intake × sugar	1	1.91	0.168
		Strain × sugar × total	2	2.78	0.065
		Error	167		
	Growth	Strain	2	2.16	0.119
		Sugar	1	8.43	0.004
		Strain × sugar	2	4.15	0.017
		Total intake	1	132.03	0.0001
		Total in × strain	2	0.67	0.512
		Total in × sugar	1	1.51	0.222
		Strain × sugar × total	2	0.74	0.480
		Error	167		
	Survival	Strain	2	6.34	0.002
		Sugar	1	14.38	0.0002
		Strain × sugar	2	8.20	0.0004
		Total intake	1	4.27	0.040
		Total in × strain	2	0.44	0.644
		Total intake × sugar	1	0.04	0.846
		Strain × sugar × total	2	1.51	0.223
		Error	168		

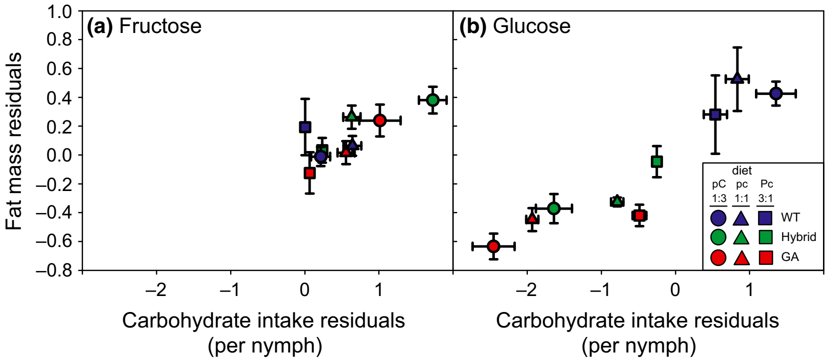

Fig. 2 Post-ingestive nutrient processing across cockroach strains and diets varying in macronutrient content and sugar type. Comparing fat mass in body tissue relative to carbohydrate intake on (a) fructose-based and (b) glucose-based diets. All mean values provided ±SEM.

among strains fed fructose-based diets (Fig. 4c,e), GA nymphs had lower dietary intake ($F_{1,163} = 152.12$, $P = 0.0001$; Table 1), growth ($F_{1,167} = 4.15$, $P = 0.017$; Fig. 4d) and survival ($F_{1,168} = 8.20$, $P = 0.0004$; Fig. 4f) on glucose-based diets relative to WT (Tukey, $P < 0.007$). Hybrid nymphs, in contrast, exhibited inter-

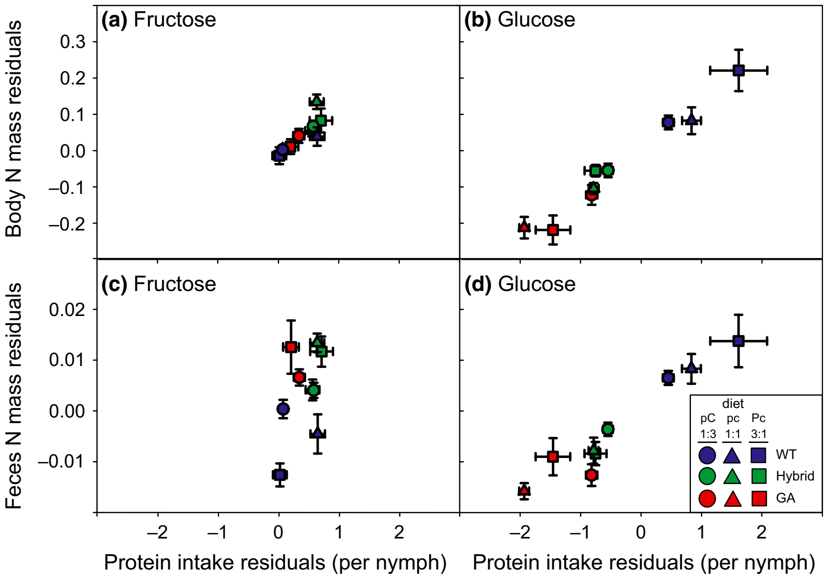

Fig. 3 Post-ingestive nutrient processing across cockroach strains and diets varying in macronutrient content and sugar type. Comparing (a,b) N (nitrogen) mass in body tissue and (c,d) N mass excreted in faeces relative to protein intake. All mean values provided ±SEM.

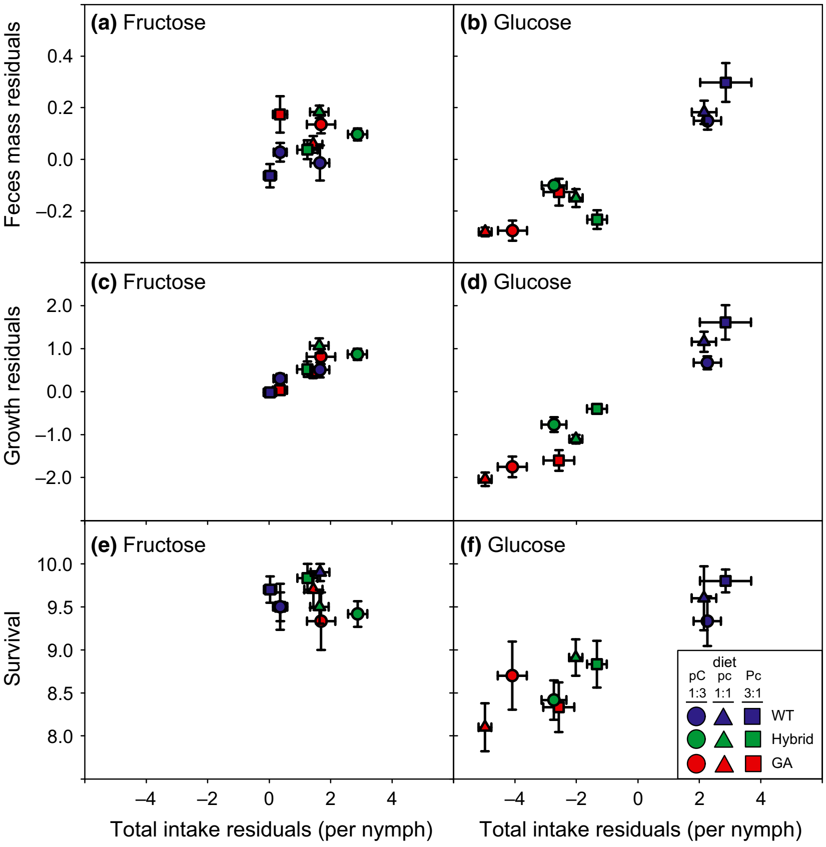

Fig. 4 Performance across cockroach strains and diets varying in macronutrient content and sugar type. Comparing rates of (a,b) growth, (c,d) excretion and (e,f) survival relative to total intake. All mean values provided ±SEM.

mediate growth and survival on glucose-based diets (Fig. 4d,f). Hybrids did not have higher growth or survival than GA nymphs (Tukey, $P > 0.17$), but hybrids also did not have lower growth than WT nymphs (Tukey, $P = 0.08$) – although they had significantly lower survival (Tukey, $P = 0.0002$).

Discussion

The rapid evolution of diet specialization in the cockroach *B. germanica* does not appear linked with compensatory strategies for nutrient regulation. First, glucose-averse (GA) nymphs had lower performance than a wild-type (WT) strain when confined to glucose-based diets, with lower carbohydrate intake and fat mass, lower protein intake and N body mass, and lower growth and survival. Second, strains exhibited similar positive relationships between dietary intake and the assimilation and excretion of nutrients, suggesting similar post-ingestive strategies for balancing nutrients. However, a GA x WT 'hybrid' had lower glucose aversion than parental GA homozygotes, consuming more of the glucose-based diets and having fat mass and growth similar to WT nymphs. Given these intermediate foraging constraints and performance costs, we thus propose that hybrids may serve as a reservoir for this maladaptive trait within populations in the absence of positive selection and account for the rapid evolution of this trait following toxic bait application.

More generally, while omnivores are thought to rely on food switching to meet nutritional requirements (Simpson & Raubenheimer, 2012), glucose-averse cockroaches provide unique opportunities to explore how an evolutionary narrowing of diet breadth shapes the outcome of nutritional trade-offs. GA nymphs had an extremely concave intake array on glucose-based diets, with their lowest intake on the pc diet that most closely matched their preferred nutritional blend. It thus appears that glucose aversion trumps diet quality when prioritizing macronutrient intake, with the concave intake array suggesting a 'maximum tolerable intake' of glucose and the expectation of compensatory foraging to locate more palatable food (Raubenheimer *et al.*, 2005). This dietary rule of compromise differs both from other insects that increasingly consume dietary toxins in high-quality diets (Simpson & Raubenheimer, 2001; Behmer *et al.*, 2002) and WT *B. germanica* whose linear intake array reflects the 'equal distance rule' typical of omnivores that ingest excess nutrients regardless of their identity (Raubenheimer & Jones, 2006; Simpson & Raubenheimer, 2012).

Glucose aversion does not appear associated with digestive strategies for balancing nutrients when dietary glucose constrains foraging towards a macronutrient intake target. For instance, GA nymphs might be expected to offset energy limitation via gluconeogenesis in specialized fat body cells called mycetocytes (Storey & Bailey, 1978). However, GA nymphs did not retain or excrete particularly large amounts of N relative to the amount of protein ingested from glucose-based diets, suggesting they were not accelerating the de-amination of amino acids to provide carbon for energy metabolism. To this, we add the caveat GA nymphs selected a higher protein:carbohydrate than WT nymphs when provided palatable, fructose-based diets in the diet choice experiment, providing some evidence of lower carbohydrate requirements. More generally, while bacterial symbionts were likely important in the evolutionary success of cockroaches (e.g. *Periplaneta americana* (Linneaus); Sabree *et al.*, 2009), helping them persist on nutritionally poor diets of detritus (Cochran, 1985), the effects of narrowed diet breadth on the evolution of cockroach microbial symbionts are ripe for further study (Sachs *et al.*, 2011).

Despite the extreme costs of glucose aversion documented in this study, the rapid parallel evolution of this trait following toxic bait application suggests the trait can nevertheless generally be present at low frequency in populations (Silverman & Ross, 1994; Wang *et al.*, 2004; Wada-Katsumata *et al.*, 2013). However, the deleterious effects of glucose aversion likely extend far beyond the nymphal stage, given the ultimate fitness costs of dietary nutrient imbalance during development of the cockroach *Nauphoeta cinerea* (Olivier) (Barrett *et al.*, 2009) and the fact that carbohydrate deficits in males can reduce sex pheromone production and thus access to *N. cinerea* females (South *et al.*, 2011). Our studies of GA heterozygotes suggest at least a partial answer to this mystery, because these hybrids exhibit higher intake than GA homozygotes on glucose-based diets and thus have lower performance costs. In addition, delayed development has been observed among WT *B. germanica* fed nutritionally imbalanced diets (Raubenheimer & Jones, 2006) and GA *B. germanica* nymphs forced on glucose-based diets (Wada-Katsumata *et al.*, 2011). Adding days between moults may help reduce the costs of glucose aversion – and promote the persistence of this trait in populations – by enabling nymphs to retain proteins that would otherwise be invested in new cuticle and carbohydrates that would fuel the energetic costs of chitin production. Exploring the costs of glucose aversion relative to life history variation in natural populations thus represents an important next step.

A remaining question is why *B. germanica* evolved a behavioural aversion to glucose rather than a physiological resistance to the bait toxicant and why aversion appears specific to glucose, rather than any other sugar. We propose that glucose aversion can be understood by turning to insights from decomposer food web ecology, whereby cockroaches typically encounter foods – plant material and organic matter colonized by microbes – containing cyanogenic glycosides, toxic secondary metabolites bound to a glucose molecule (Janzen, 1977; Coley *et al.*, 1985). We propose this selects for glucose sensitivity, enabling cockroaches (and potentially other decomposer organisms) to avoid the most toxic foods. The evolution of glucose aversion is somewhat puzzling given that *B. germanica* in other populations have overcome nutrient–toxin trade-offs by evolving physiological resistance to toxicants used in baits (Schal, 1992). And yet, the status of cockroaches as extreme omnivores that can simply forage across diverse foods (Raubenheimer & Jones, 2006) may help explain why they would use glucose to detect the toxic moiety rather than post-ingestive detoxification. Moreover, glucose aversion may provide a route to toxicant resistance if it causes individuals to repeatedly ingest small, sublethal doses – although this would likely depend on the lethality of the toxic compound (Silverman & Liang, 1999). As these questions are answered, glucose-averse cockroaches will provide much broader insights into co-evolutionary interactions between plants and insects, and strategies used by microbes (e.g. food moulding) to prevent ingestion of colonized food by animals.

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References

Agrawal, A.A. & Klein, C.N. 2000. What omnivores eat: direct effects of induced plant resistance on herbivores and indirect consequences for diet selection by omnivores. *J. Anim. Ecol.* **69**: 525–535.

Barrett, E.L.B., Hunt, J., Moore, A.J. & Moore, P.J. 2009. Separate and combined effects of nutrition during juvenile and sexual development on female life-history trajectories: the thrifty phenotype in a cockroach. *Proc. R. Soc. B* **276**: 3257–3264.

Behmer, S.T., Simpson, S.J. & Raubenheimer, D. 2002. Herbivore foraging in chemically heterogeneous environments: nutrients and secondary metabolites. *Ecology* **83**: 2489–2501.

Cochran, D.G. 1985. Nitrogen excretion in cockroaches. *Annu. Rev. Entomol.* **30**: 29–49.

Coley, P.D., Bryant, J.P. & Chapin, F.S. 1985. Resource availability and plant antiherbivore defense. *Science* **230**: 895–899.

Dussutour, A. & Simpson, S.J. 2008. Description of a simple synthetic diet for studying nutritional responses in ants. *Ins. Soc.* **55**: 329–333.

Freeland, W.J. & Janzen, D.H. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. *Am. Nat.* **108**: 269–289.

Janzen, D.H. 1977. Why fruits rot, seeds mold, and meat spoils. *Am. Nat.* **980**: 691–713.

Jones, S.A. & Raubenheimer, D. 2001. Nutritional regulation in nymphs of the German cockroach, *Blattella germanica*. *J. Insect Physiol.* **47**: 1169–1180.

Kay, A.D., Shik, J.Z., Van Alst, A. & Kaspari, M. 2012. Diet composition does not affect ant colony tempo. *Funct. Ecol.* **26**: 317–323.

Lee, K.P., Behmer, S.T. & Simpson, S.J. 2006. Nutritional regulation in relation to diet breadth: a comparison of *Heliothis* sister species and a hybrid. *J. Exp. Biol.* **209**: 2076–2084.

Mullins, D.E. & Keil, C.B. 1980. Parental investment of urates in cockroaches. *Nature* **283**: 567–569.

Rapport, D.J. 1980. Optimal foraging for complementary resources. *Am. Nat.* **116**: 324–346.

Raubenheimer, D. 1992. Tannic acid, protein, and digestible carbohydrate: dietary imbalance and nutritional compensation in locusts. *Ecology* **73**: 1012–1027.

Raubenheimer, D. & Jones, S.A. 2006. Nutritional imbalance in an extreme generalist omnivore: tolerance and recovery through complementary food selection. *Anim. Behav.* **71**: 1253–1262.

Raubenheimer, D., Zemke-White, W.L., Phillips, R.J. & Clements, K.D. 2005. Algal macronutrients and food selection by the omnivorous fish *Girella tricuspidata*. *Ecology* **86**: 2601–2610.

Ross, M.H. & Silverman, J. 1995. Genetic studies of a behavioral mutant, glucose aversion, in the German cockroach. *J. Insect Behav.* **8**: 825–834.

Sabree, Z.L., Kambhampati, S. & Moran, N.A. 2009. Nitrogen recycling and nutritional provisioning by *Blattabacterium*, the cockroach endosymbiont. *Proc. Natl. Acad. Sci. USA* **106**: 19521–19526.

Sachs, J.L., Skophammer, R.G. & Regus, J.U. 2011. Evolutionary transitions in bacterial symbiosis. *Proc. Natl. Acad. Sci. USA* **108**: 10800–10807.

Schal, C. 1992. Sulfuramid resistance and vapor toxicity in field-collected German cockroaches (Dictyoptera: Blattellidae). *J. Med. Entomol.* **29**: 207–215.

Schal, C., Gautier, A.-S. & Bell, W.J. 1984. Behavioural ecology of cockroaches. *Biol. Rev.* **59**: 209–254.

Silverman, J. 1995. Effects of glucose-supplemented diets on food intake, nymphal development, and fecundity of glucose-averse, non-glucose-averse, and heterozygous strains of the German cockroach, *Blattella germanica*. *Ent. Exp. Applic.* **76**: 7–14.

Silverman, J. & Bieman, D.N. 1993. Glucose aversion in the German cockroach *Blattella germanica*. *J. Insect Physiol.* **39**: 925–933.

Silverman, J. & Liang, D. 1999. Effect of fipronil on bait formulation-based aversion in the German cockroach (Dictyoptera: Blattellidae). *J. Econ. Entomol.* **92**: 886–889.

Silverman, J. & Ross, M.H. 1994. Behavioral resistance of field-collected German cockroaches (Blattodea: Blattellidae) to baits containing glucose. *Environ. Entomol.* **23**: 425–430.

Simpson, S.J. & Raubenheimer, D. 2001. The geometric analysis of nutrient-allelochemical interactions: a case study using locusts. *Ecology* **81**: 422–439.

Simpson, S.J. & Raubenheimer, D. 2012. *The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity*. Princeton University Press, New Jersey.

Smith, C.R. & Tschinkel, W.R. 2009. Ant fat extraction with a soxhlet extractor. *Cold Spring Harb. Prot.* **4**: 832–835.

South, S.H., House, C.M., Moore, A.J., Simpson, S.J. & Hunt, J. 2011. Male cockroaches prefer a high carbohydrate diet that makes them more attractive to females: implications for the study of condition dependence. *Evolution* **65**: 1594–1606.

Storey, K.B. & Bailey, E. 1978. Intracellular distribution of enzymes associated with lipogenesis and gluconeogenesis in fat body of the adult cockroach *Periplaneta*. *Insect Biochem.* **8**: 125–131.

Trier, T.M. & Mattson, W.J. 2003. Diet induced thermogenesis in insects: a developing concept in nutritional ecology. *Environ. Entomol.* **32**: 1–8.

Wada-Katsumata, A., Silverman, J. & Schal, C. 2011. Differential inputs from chemosensory appendages mediate feeding responses to glucose in wild-type and glucose-averse German cockroaches, *Blattella germanica*. *Chem. Sens.* **36**: 589–600.

Wada-Katsumata, A., Silverman, J. & Schal, C. 2013. Changes in taste neurons support the emergence of an adaptive behavior in cockroaches. *Science* **340**: 972–975.

Wang, C., Scharf, M. & Bennett, G.W. 2004. Behavioral and physiological resistance of the German cockroach to gel baits (Dictyoptera: Blattellidae). *J. Econ. Entomol.* **97**: 2067–2072.

Warbrick-Smith, J., Raubenheimer, D., Simpson, S.J. & Behmer, S.T. 2009. Three hundred and fifty generations of extreme food specialisation: testing predictions of nutritional ecology. *Ent. Exp. Applic.* **132**: 65–75.

Zanotto, F.P., Simpson, S.J. & Raubenheimer, D. 1993. The regulation of growth by locusts through post-ingestive compensation for variation in the levels of dietary protein and carbohydrates. *Physiol. Entomol.* **18**: 425–434.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Preparation of synthetic diet.

Table S1 Hybrid analyses: treatment responses in reciprocally crossed GA × WT hybrid strains.

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