J. exp. Biol. **162**, 55–72 (1992)
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MATERNAL AND PATERNAL NITROGEN INVESTMENT IN *BLATTELLA GERMANICA* (L.) (DICTYOPTERA; BLATTELLIDAE)

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Accepted 6 August 1991

Summary

The investment of nitrogenous materials by female and male German cockroaches *Blattella germanica* (L.) into their progeny was examined. Adult females maintained on dog food invested 34 % of their dry mass and 26 % of their nitrogen into an ootheca during their first gonadotrophic cycle. Females maintained on a low- (5 %) protein diet and injected simultaneously with [^{3}H]leucine and [^{14}C]hypoxanthine incorporated less [^{3}H]leucine-derived radiolabel in their oothecae than those on a dog food diet (25 % crude protein). Females on the low-protein diet incorporated more [^{14}C]hypoxanthine-derived material (primarily as [^{14}C]urates) into their oothecae than they retained in their bodies. Stored [^{14}C]urates were metabolized more readily by females on the low-protein diet. Oothecae obtained from females provided with an [^{15}N]urate-amended diet contained at least four ^{15}N-enriched amino acids, which supports the hypothesis that urates are utilized as a nitrogen resource in these insects.

Dietary effects on paternal investment were also found to be significant. Females fed a low-protein diet and their oothecae contained 63 % of the radiolabel made available to them at mating when paired with males injected simultaneously with [^{3}H]leucine and [^{14}C]hypoxanthine, whereas dog-food-fed females and their oothecae contained only 17 % of the total radiolabel made available to them at mating.

Introduction

Insects utilize a variety of strategies and adaptations to fulfil nutritional

Key words: *Blattella germanica*, parental investment, nitrogen metabolism.

requirements for their growth, development and reproduction (Thornhill and Gwynne, 1986; Slansky and Rodriguez, 1987). Reproductive strategies among cockroaches are diverse and may include oviparity, ovoviviparity or viviparity (Roth, 1968; Stay and Coop, 1974), prezygotic investment at mating by the male (Mullins and Keil, 1980; Schal and Bell, 1982) and maternal or biparental care (Nalepa, 1988). Cockroaches also possess a considerable amount of latitude in their food selection (Lembke and Cochran, 1989), partly because of their ability to mobilize stored fat-body urates. The mobilization of these internally stored fat-body urates is probably achieved by bacterial symbionts, which provide a resource of nitrogen-containing materials for these insects (Mullins and Cochran, 1987; Wren and Cochran, 1987; De Piceis Polver *et al.* 1986) and allow them to survive on a variety of food materials, including those that may be low in nitrogen (Cochran, 1985). The dietary requirements of reproducing cockroaches indicate that dietary nitrogen may influence fecundity (Melampy and Maynard, 1937; Haydak, 1953; Gordon, 1959; Kunkel, 1966; Mullins and Cochran, 1975*a*,*b*; Cochran, 1983; Durbin and Cochran, 1985; Hamilton and Schal, 1988; Hamilton *et al.* 1990). It has also been reported that uric acid may be transferred from males to females at mating, presumably to support the nutritional requirements of oogenesis and/or embryogenesis (Mullins and Keil, 1980; Schal and Bell, 1982). To evaluate dietary influences on parental nitrogen investment into German cockroach progeny, two radiolabelled materials; purines (as [^{14}C]hypoxanthine) and an amino acid (as [^{3}H]leucine) were injected simultaneously into either females or males maintained on two different dietary nitrogen levels. Dual radiolabel techniques were used to determine incorporation, retention and transfer of radiolabel between males and females and their progeny. In a separate experiment, a stable nitrogen isotope (^{15}N) of uric acid was provided in a diet to determine the fate of urate nitrogen in ootheca-producing females.

Materials and methods

German cockroaches used in this study were obtained from established cultures held at approximately 25 °C and ambient humidity in 4 l glass battery jars and provided with dog food (25 % crude protein) and water *ad libitum*. The sexes were separated at a late nymphal stage and held in groups until eclosion in 1 l glass jars also provided with food and water. At adult eclosion, single females were placed in 10 cm×2 cm plastic Petri dishes fastened to the top of a 1.8 g vial containing distilled water. A glass tube (0.3 cm×7 cm) packed with a cotton wick was inserted through the top of the vial and the bottom of the Petri dish, providing access to water. The diets used were: a 5 % protein diet described previously (Mullins, 1974), a 5 % protein diet containing 10 % [^{15}N]uric acid, or dog food (25 % crude protein). They were dispensed into small containers, packed, steamed (10 min) and oven-dried (80 °C for 1 h) prior to presentation to the insects. Older nymphs and adults were held in an environmental chamber (28±2 °C, 55–65 % relative humidity). Data were analyzed using the Student's *t*-test (Sokal and Rohlf, 1981).

Radiolabelled isotope experiments

A motor-driven microapplicator was used to inject 1 μl volumes of radiolabelled [^{3}H]leucine (L-[4,5-^{3}H]leucine, specific activity 52 Ci mmol^{-1}; ICN-Pharmaceuticals) and [^{14}C]hypoxanthine ([8-^{14}C]hypoxanthine, specific activity 53.4 mCi mmol^{-1}; ICN-Pharmaceuticals) into the insect's abdomen. Radiolabelled hypoxanthine was used as the urate (purine) source instead of xanthine or uric acid because its greater solubility in aqueous solutions facilitated the injection of relatively equivalent amounts into each insect. The subsequent *in vivo* conversion of hypoxanthine to uric acid is rapid and quantitative (Mullins and Keil, 1980; Cochran and Mullins, 1982; Engebretson and Mullins, 1983, 1986).

At the conclusion of radiolabel experiments, insects were frozen and whole bodies and oothecae were homogenized in 1 ml of 0.6 % Li_2CO_3 (80 °C for 10 min) and centrifuged at 1000 *g* (10 min). Samples of extracts (250–500 μl) were placed into 8 ml phase-combining system (PCS) (Amersham Searle)/xylene (1:1) scintillation cocktail. Separation and quantification of radiolabelled urate were by thin-layer chromatography (TLC) on 1 mm cellulose plates (20 cm×20 cm) developed in *n*-butanol/methanol/water/NH_4OH (60:20:20:1) and scintillation counting of the recovered and extracted urate spots, respectively. Total urate was also determined by the uricase method (Dubbs *et al.* 1956) as modified by Mullins and Cochran (1976). Additional details of the three radiolabel experiments are provided in table footnotes.

Stable isotope experiment

Newly eclosed adult females were provided with a 5 % protein diet containing 10 % [^{15}N]uric acid ([1,3-^{15}N]uric acid, 95 atom%; Merck & Co.). When 19 mg of this diet had been consumed, insects were placed on either a dog food or a 5 % protein diet, and were provided with an opportunity to mate (placed with two males for 1 h daily). Oothecae were removed and frozen 21 days after they appeared. Because of the expense of analysis, only one female and her ootheca from each diet were analyzed. The presence of ^{15}N in the amino acids in the oothecae was determined by hydrolysis with 6 mol l^{-1} HCl under N_2 at 110 °C for 24 h. The hydrolysate was evaporated to dryness with a stream of N_2, dissolved in water, filtered and again evaporated to dryness under a stream of nitrogen. The resulting crude amino acids were converted to *n*-butyl esters by heating for 3 h at 100 °C with 3 mol l^{-1} HCl in *n*-butanol. After removing the HCl and *n*-butanol by evaporation under a stream of nitrogen, the resulting amino acids were converted to their trifluoroacetic acid derivatives by reaction for 12 h with a mixture of trifluoroacetyl anhydride and methylene chloride (1:1 v/v). Evaporation of the solvent left a brown residue which was mixed with 3 mol l^{-1} $NaHCO_3$, extracted with heptane and dried over Na_2SO_4. The presence of ^{15}N in the individual amino acid derivatives contained in the concentrated heptane solution was determined by gas chromatography–mass spectroscopic analysis, as previously described (White, 1985).

Nitrogen and uric acid determinations

Determinations of whole-body and oothecal nitrogen and urates in non-radiolabelled insects were carried out on lyophilized tissue pulverized using a dental amalgamator and liquid nitrogen. Total nitrogen was determined using a micro-Kjeldahl procedure and urate content was estimated using a spectrophotometric uricase assay (Mullins and Cochran, 1976).

Results

Maternal investment experiments

The total mass, the nitrogen content and the urate nitrogen content of reproducing females were obtained to provide information on female allocation of nutritional resources into progeny. Dog-food-fed females plus their newly eclosed nymphs and emptied oothecae had a mean mass of 36.5±2.1 mg and contained a total of 235±49 μmol nitrogen (Table 1). Urate nitrogen accounted for 51 % (females) and 6.4 % (nymphs) of the total nitrogen (Table 1).

Radiolabelled leucine was injected into females 0–2 or 5–7 days after imaginal eclosion to determine the effects of diet and time of injection on leucine-derived radiolabel incorporation into newly formed oothecae. Females on both diets retained more radiolabel when they were injected 0–2 days after imaginal eclosion (Table 2). Radiolabel incorporation into oothecae by females maintained on the dog food diet and injected 5–7 days post-imaginal eclosion was greater than that of females injected at 0–2 days, but the difference for females fed the 5 % protein diet was not significant. Similarly, differences were observed in total radiolabel retention in dog-food-fed females and their oothecae injected at either 0–2 or 5–7 days, but not in the females fed a 5 % protein diet.

Table 1. *Distribution of mass, nitrogen and urate nitrogen in female* Blattella germanica *and their newly hatched oothecae (dog food diet)*

	Dry mass		Nitrogen content		Urate nitrogen content	
	(mg)	(%)	(μmol)	(%)	(μmol)	(% of total N_2)[1]
Females	24.0±0.7	66	173±2	74	88±6.5	51
Newly emerged nymphs	10.9±3.1	30	52±5	22	3.9±0.41	7.5
Empty oothecae	1.6±0.1	4	9±1	4	ND	–
Nymphs+oothecae	12.5±0.7	34	61±5	26	3.9±0.41	6.4

Values are expressed as the mean±standard error (N=5). The percentage dry mass and nitrogen content are based on the totals of the female+nymphs+empty oothecae (mass= 36.5±2.1 mg; nitrogen content=235±49 μmol).

[1] Percentage of total nitrogen in the respective groups.

ND, not detectable.

label incorporation into females and their oothecae injected 0–2 and 5–7 days post-imaginal eclos
[^{3}H]leucine maintained on either dog food or 5 % protein diets

	Diet[1,2]						
	Dog food				5 % protein		
[3]	Female (disints min^{-1})[4]	Oothecae (disints min^{-1})	Total (disints min^{-1})	*N*	Female (disints min^{-1})	Oothecae (disints min^{-1})	(disin
	50 107±2765[a]	24 146±870[b]	74 253±3344[c]	10	44 920±1710[d]	28 915±1762[e]	73 83
	38 %	18 %	56 %		34 %	22 %	
6	26 651±5419[A]	65 213±3623[B]	91 864±4768[C]	8	26 775±2292[D]	38 387±5463[E]	65 16
	20 %	49 %	69 %		20 %	29 %	

nymphs were placed on 5 % protein or dog food (25 % crude protein) diets 7 days prior to injec
sints min^{-1} [^{3}H]leucine in 1 μl of distilled H_2O. After injection, females were placed in separate cages along with tw
unity to mate. Females and their oothecae were frozen separately when oothecae appeared.
o produce an ootheca: dog food diet=14.3 days post-imaginal eclosion; 5 % protein diet=15.3 days post-imaginal
significantly different (P=0.183), Student's t-test.
insects.
rd error of the mean; percentages were calculated on the basis of injected dose.
parisons: Student's t-test: NS, means not significantly different; S, means significantly different. a/d, NS (P=0.13
NS (P=0.881); A/D, NS (P=0.970); B/E S (P=0.002); C/F, S (P=0.015); c/C, S (P=0.007); f/F, NS (P=0.25
3, S (P=0.000008); d/D, S (P=0.000008); e/E, NS (P=0.09).

Several observations can be made regarding diet effects on [^{3}H]leucine retention/incorporation. Females injected 0–2 days after imaginal eclosion and maintained on either diet retained similar amounts of radiolabel. However, more radiolabel was incorporated into oothecae by females maintained on the low-protein (5 %) diet. Females on both diets injected 5–7 days after imaginal eclosion also retained similar levels of radiolabel, but more radiolabel was incorporated into oothecae by females maintained on the higher-protein (dog food) diet.

A dual-label experiment was conducted using two metabolically different nitrogen-containing resources to examine the effects of diet on their incorporation during oocyte development to maturation. [^{14}C]Hypoxanthine was used to examine incorporation, translocation and metabolism of urate and [^{3}H]leucine was used to assess the incorporation and metabolism of a representative amino acid (Table 3). The time required to produce an ootheca by females maintained on the 5 % protein diet was significantly longer than that required for females on the dog food diet (42.2 and 13.9 days, respectively), indicating that lower dietary nitrogen levels, beginning 14 days prior to eclosion but not 7 days prior to eclosion, affected the rate of oothecal production (Table 2, footnote).

Females maintained on dog food retained 25 % of the injected [^{3}H]leucine-derived radiolabel in their bodies and invested 31 % into their oothecae (Table 3). Total retention of the radiolabel to the time of oothecal production was 56 %. Females maintained on the 5 % protein diet retained a similar amount of radiolabel in their bodies but significantly less radiolabel was incorporated into their oothecae (17 %). This lower rate of leucine incorporation suggests that more of the amino acid was metabolized by females as a result of their maintenance on the lower-protein diet.

The fate of radiolabelled hypoxanthine also indicates that differences in allocation occurred in response to dietary regimen. Females on dog food retained 65 % of the injected dose as [^{14}C]hypoxanthine-derived radiolabel and invested 29 % in their oothecae (Table 3). Females maintained on 5 % protein retained significantly less radiolabel (12 %) but invested a similar amount of radiolabel into their oothecae (41 %). Total hypoxanthine-derived radiolabel retention was higher in the dog-food-fed females and their oothecae than in those maintained on the lower protein diet.

Additional information on the fate of urates in the dual-label experiment is provided in Table 4. In the females fed dog food, 98 % of the hypoxanthine-derived radiolabel remaining in their bodies was [^{14}C]urate, as was 84 % of the radiolabel incorporated into their oothecae. Those females maintained on 5 % protein also retained 98 % of the hypoxanthine-derived radiolabel in their bodies as [^{14}C]urate, but only 75 % of the radiolabel incorporated into their oothecae was [^{14}C]urate. Females on the dog food diet contained over four times as much urate as did those on the 5 % protein diet. Oothecae from females fed dog food also contained more urate than those on the 5 % protein diet. Significant quantities of urates were incorporated into oothecae during oogenesis/oothecal production.

[^{15}N]Uric acid was fed to females on dog food and 5 % protein diets to obtain

Table 3. *Radiolabel content of female* Blattella germanica *and their oothecae after simultaneous injection of [^{3}H]leucine and [^{14}C]hypoxanthine into virgin females followed by mating and oothecal production while feeding on either dog food or 5 % protein diets*

Diet	N[1]	Oothecal formation: days after imaginal eclosion	^{14}C content derived from [^{14}C]hypoxanthine			^{3}H content derived from [^{3}H]leucine		
			Female (disints min^{-1})[2]	Oothecae (disints min^{-1})	Total (disints min^{-1})	Female (disints min^{-1})	Oothecae (disints min^{-1})	Total (disints min^{-1})
Dog food	9	13.9±0.6[a]	53 788±2928[c]	23 481±3309[e]	77 269±2446[g]	79 828±9265[C]	98 928±6521[E]	178 756±9379[G]
			65 %	29 %	94 %	25 %	31 %	56 %
5 % protein	12	42.2±6.2[b]	10 093±2762[d]	34 156±8611[f]	44 249±7681[h]	73 898±5140[D]	54 703±9353[F]	128 601±12 152[H]
			12 %	41 %	53 %	23 %	17 %	40 %

Late instar nymphs were placed on 5 % or dog food (25 % crude protein) diets 14 days prior to imaginal eclosion. Adult females were injected simultaneously with 1 μl of H_2O containing 318 841±26 362 disints min^{-1} [^{3}H]leucine and 82 419±7933 disints min^{-1} [^{14}C]hypoxanthine at 5–6 days post-imaginal eclosion. After injection, females were placed in cages along with two males to ensure an opportunity to mate. Females and their oothecae were both frozen (separately) when the oothecae appeared.

[1] N, number of insects.

[2] Mean±standard error; percentages were calculated on the basis of injected dosage.

Statistical comparisons: Student's t-test: NS, means not significantly different; S, means significantly different; a/b, S (P=0.0008); c/e, S (P=0.013); c/d, S (P=0.000001); d/f, S (P=0.000004); e/f, NS (P=0.31); g/h, S (P=0.002); C/D, NS (P=0.56); E/F, S (P=0.002); G/H, S (P=0.005).

Table 4. *Radiolabelled urate and total urate content of female* Blattella germanica *and their oothecae after simultaneous injection of [^{3}H]leucine and [^{14}C]hypoxanthine into virgin females followed by mating and oothecal production while feeding on either dog food or 5 % protein diets*

		Total [^{14}C]urate content		Total urate content	
Diet	*N*	Female (disints min^{-1})[1]	Oothecae (disints min^{-1})	Female (μmol)	Oothecae (μmol)
Dog food	9	52 773±2075[a] 98 %[2]	19 662±2315[c] 84 %	9.9±0.8[e] 80 %[3]	2.4±0.1[g] 20 %
5 % protein	12	9875±2534[b] 98 %	25 753±7207[d] 75 %	2.1±0.4[f] 53 %	1.9±0.3[h] 47 %

Experimental protocol provided in footnotes to Table 3.

[1] Means±standard error; percentages were calculated on the basis of injected dosage.

[2] Percentage of total ^{14}C radiolabel appearing as [^{14}C]urate.

[3] Percentage of total urate in females or their oothecae based on 12.3 μmol (dog-food-fed females) and 4.0 μmol (5 % protein-diet-fed females).

Statistical comparisons: Student's *t*-test: NS, means not significantly different; S, means significantly different; a/b, S (P=0.000001); c/d, NS (P=0.48); e/f, S (P=0.000001); g/h, S (P=0.002).

preliminary information on the fate of uric acid nitrogen during oothecal production and embryonic development. Both 21-day-old oothecae contained the following ^{15}N-labelled amino acids: alanine, proline, glutamic acid and aspartic acid. Comparison of the relative amounts of these amino acids indicates that the ootheca obtained from the female maintained on the 5 % protein diet contained more of the ^{15}N label (Table 5).

Paternal investment experiment

A dual radiolabel protocol using [^{14}C]hypoxanthine and [^{3}H]leucine was employed to assess the incorporation of radiolabel injected into males and subsequently transferred to females and their oothecae after mating. As in the maternal investment dual-label experiment, [^{14}C]hypoxanthine was used as an indicator of the transfer of urate-derived metabolites and [^{3}H]leucine was used as an indicator of the transfer of leucine (amino acid)-derived metabolites to females and their oothecae. Males injected with the two radiolabelled compounds were paired with females and the distribution of radiolabel was determined after an ootheca had been produced. The times required to produce one ootheca by females maintained on dog food prior to pairing, but placed on either dog food or 5 % protein diets at pairing, were similiar (Table 6, footnote). Male radiolabel retention is indicated in two categories (mated and unmated). Those males from each replicate group (2 males and 1 female) that had the lower ^{14}C content of the pair were designated as having mated, since radiolabel contained in the accessory gland as [^{14}C]urate is voided at mating with the spermatophore. Correspondingly,

Table 5. *Incorporation of ^{15}N into oothecae (as amino acids) obtained from female* Blattella germanica *fed [^{15}N]uric acid and maintained on either dog food or 5 % protein diets*[1]

			Labelled samples			
			Dog food diet		5 % protein diet	
Amino acid	m/z^2 of the ion used in the measurement	Unlabelled sample [intensity of (ion+1m/z)]/ [intensity of (ion)]	Measured [intensity of (ion+1m/z)]/ [intensity of (ion)]	Mole% excess ^{15}N[3]	Measured [intensity of (ion+1m/z)]/ [intensity of (ion)]	Mole% excess ^{15}N
Alanine	140	36.1	42.2	5.7	46.2	9.2
Proline	166	8.1	11.9	3.6	16.7	7.9
	276	15.3	18.4	3.0	24.1	8.1
Glutamic acid	180	11.9	16.4	4.3	20.2	7.7
	198	7.5	13.0	5.2	17.2	8.8
Aspartic acid	240	38.5	38.7	<0.2	42.4	3.8

[1] Newly eclosed adult females were provided with 19 mg of 5 % protein diet containing 10 % [^{15}N]uric acid (uric acid 1,3-^{15}N; 95 atom%). When this diet was consumed (6.3±0.6 days) females were placed on either a dog food (25 % crude protein) or 5 % protein diet. Each day, two similarly aged, dog-food-fed males were provided with an opportunity to mate (1 h) with the females. Mating occurred at 11 days (dog-food-fed female) and 17 days (5 % protein-diet-fed female) post-imaginal eclosion. Oothecae appeared 15 days (dog-food-fed females) and 20 days (5 % protein-diet-fed females) after imaginal eclosion, and were removed and frozen 21 days after females had produced them.

[2] Mass to charge ratio.

[3] Calculated from the difference between the ratios of (ion+1m/z)/(ion) for labelled and unlabelled samples.

Table 6. *Radiolabel content after simultaneous injection of [^{3}H]leucine and [^{14}C]hypoxanthine into virgin males followed by mating with females and oothecal production while feeding on either dog food or 5 % protein diets*

		Radioactivity[2]								
		disints min^{-1}/(% injected dose)								% Injected dose
		Males[3]								
Diet	Radiolabel[1]	Unmated		Mated		Females		Oothecae		Female+oothecae
Dog food	[^{14}C]Hypo	73911±3093	(88 %)^A	58796±6046	(70 %)^B	1083±457	(1.3 %)^E	532±317	(0.6 %)^G	(1.9)^I
	[^{3}H]Leu	108518±10908	(35 %)^a	94244±8025	(31 %)^b	1690±282	(0.5 %)^e	1686±443	(0.5 %)^g	(1.0)^i
5 % protein	[^{14}C]Hypo	68202±4414	(81 %)^C	39948±3469	(47 %)^D	1516±559	(1.8 %)^F	5516±2205	(6.5 %)^H	(8.3)^J
	[^{3}H]Leu	109597±9917	(35 %)^c	108217±6686	(35 %)^d	5576±868	(1.8 %)^f	6003±1665	(1.9 %)^h	(3.7)^j

[1] Dog-food-fed males (1 week post-imaginal eclosion) were injected with 309257±26633 disints min^{-1} [^{3}H]leucine ([^{3}H]Leu) and 84364±9427 disints min^{-1} [^{14}C]hypoxanthine ([^{14}C]Hypo) in 1 μl of distilled H_2O. Twenty days post-injection, two males were placed with dog-food-fed females (2 days after imaginal eclosion) in separate cages containing either dog food (25 % crude protein) or 5 % protein diets. There were five replicate groups (two males+one female) placed on each diet. All females produced oothecae: dog food diet fed at 21.6±1.7 days after imaginal eclosion; 5 % protein diet fed at 21.0±0.7 days after imaginal eclosion. Means were not significantly different (P=0.727) using Student's t-test. Females, their oothecae and males were frozen separately 2 days after each ootheca appeared.

[2] Mean±standard error of the mean; percentages were calculated on the basis of injected dose.

[3] Males are segregated arbitrarily, into 'unmated' *vs* 'mated' categories on the basis of whole-body ^{14}C radioactivity, since large amounts of urates can be stored in males and significant amounts are transferred at mating (Mullins and Keil, 1980). Young females mate only once prior to producing their first ootheca.

Statistical comparisons: Student's t-test: NS, means not significantly different; S, means significantly different. A/B, S (P=0.038); C/D, S (P=0.0005); A/C, NS (P=0.27); B/D, S (P=0.016); E/F, NS (P=0.52); G/H, S (P=0.037); I/J, S (P=0.026); a/b, NS (P=0.27); c/d, NS (P=0.090); a/c, NS (P=0.94); b/d, NS (P=0.173); e/f, S (P=0.001); g/h, S (P=0.023); i/j, S (P=0.002).

those males that had the higher ^{14}C content were considered to have remained unmated.

Unmated males maintained on either diet retained significantly more ^{14}C radiolabel than mated males (Table 6). No differences in ^{14}C content between the unmated males on either of the diets were observed but dog-food-fed mated males retained more ^{14}C radiolabel than 5 %-protein-fed mated males. Females on both diets retained less than 2 % of the ^{14}C radiolabel injected into the males. However, the oothecae of the 5 %-protein-fed females contained over 10 times more than the dog-food-fed females of the ^{14}C radiolabel provided by the males. Total female and oothecal ^{14}C radiolabel acquisition values indicate that less than 2 % of the radiolabel injected into males was contained in dog-food-fed females and their oothecae whereas over 8 % of the radiolabel injected into males was contained in 5 %-protein-fed females and their oothecae.

Unmated and mated males maintained on either of the two diets retained similar amounts of ^{3}H radiolabel ranging from 31 to 35 % of the injected dose (Table 6). Differences were found in ^{3}H content of females maintained on the two diets (dog food diet 0.5 %, 5 % protein diet 1.8 % of the radiolabel injected into the males). Similar differences were observed in the ^{3}H content of oothecae obtained from these females. Females maintained on the 5 % protein diet contained more than three times as much ^{3}H in their bodies and oothecae as those maintained on the dog food diet.

Fig. 1 provides comparisons of the total ^{14}C- and ^{3}H-derived radiolabel retention in the males and acquisition by the females maintained on the two diets. Differences in total ^{14}C and ^{3}H radiolabel retention between unmated and mated males were used to estimate the amount of total radiolabel ($^{14}C+^{3}H$) that was made available to females by males during mating. Percentages in this figure represent the relative amount of material that females acquired and retained from males during mating. Comparison of total female ^{14}C and ^{3}H content in disints min^{-1} demonstrates that those females maintained on the 5 % protein diet retained more of the radiolabel than those females maintained on dog food. The amount of oothecal radiolabel content obtained from the male shows a striking difference between diets (5 % protein diet 39 %; and dog food diet 8 %). All of the radiolabel found in mated females and their oothecae was contributed by the mated males (5 % protein diet 63 %, dog food 17 %).

Discussion

Parental investment generally does not scale linearly with species mass because larger species invest less material into their progeny relative to their body mass per unit time (Reiss, 1985). Kunkel (1966) has compared reproductive potential in two cockroach species, reporting that female *Periplaneta americana* (larger species; live mass approximately 1200 mg) diminished their food reserves by 15 % with each ootheca produced, but female *B. germanica* (live mass approximately 105 mg) used up almost all (90 %) of their food reserves accumulated during the

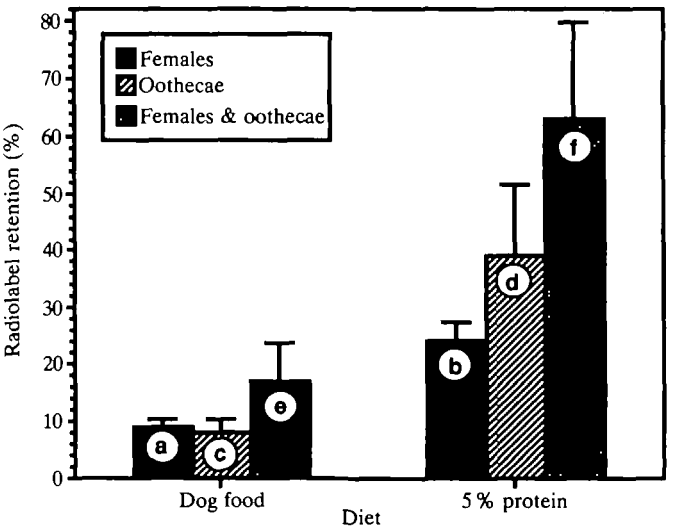

Fig. 1. Comparison of total radiolabel content after simultaneous injection of [^{3}H]leucine and [^{14}C]hypoxanthine into virgin *Blattella germanica* males followed by mating with females and their oothecae produced while feeding on either dog food or 5 % protein diets. The values are based on total ^{3}H and ^{14}C contained in these insects. The percentages were derived from differences in total radiolabel observed between the unmated and mated males. The difference was assumed to have been made available to the females at mating. See Table 6 for details. Values are mean+S.E.M. (N=5). Statistical comparisons: Student's t-test: S, means significantly different. a/b, S (P=0.005); c/d, S (P=0.027); e/f, S (P=0.007).

preovipositional period on a single ootheca. We found that significant amounts of the dry mass (34 %) and of the nitrogen content (26 %) of ootheca-carrying female *B. germanica* were contained in their newly eclosed oothecae (Table 1).

Insect nutritional status is known to affect reproductive performance (McCaffery, 1975; Slansky, 1980; Slansky and Rodriquez, 1987). Food deprivation in *B. germanica* causes a delay in the reproductive cycle (Kunkel, 1966; Mueller, 1978; Durbin and Cochran, 1985). During the preovipositional period, Cochran (1983) found that feeding and drinking in *B. germanica* females was at a high level. This fell abruptly at oviposition, with females feeding and drinking only sparingly while carrying an ootheca. Food quality has been shown to influence diet consumption. Female *B. germanica* compensate for low dietary nitrogen content (5 % protein diet) by consuming more of the diet (Hamilton and Schal, 1988).

We found that the time between post-imaginal eclosion and oothecal formation increased when late instar nymphs were placed on low-protein diets for over 7 days. Late instar nymphs placed on the 5 % protein diet 7 days before imaginal eclosion showed no dietary differences in the time required for oothecal production. However, females placed on the 5 % protein diet 14 days before imaginal eclosion required more time for oothecal production. Hamilton and Schal (1988) found no differences in the time required for oothecal formation

between females placed on a 5 % protein diet and those fed on rat food (23 % protein) at imaginal eclosion. However, another cockroach species (*Supella longipalpa*) maintained on low-protein diets from the nymph to adult stage required more time to produce an ootheca (Hamilton *et al.* 1990). Prolonged maintenance of pre-imaginal nymphs on low-protein diets appears to delay oothecal formation.

Vitellins and urates are two nitrogen-containing materials available during development of *B. germanica* embryos. Females provision their eggs with vitellins during oocyte development, providing a major amino acid resource for embryogenesis. Their utilization is correlated with embryonic growth (Purcell *et al.* 1988). Leucine is a major vitellin constituent, representing 8.7 mol% of the amino acid content in the vitellogenin precursor (Kunkel and Pan, 1976). Information obtained from [^{3}H]leucine injection into females (Tables 2 and 3) indicates that females maintained on different dietary nitrogen levels retained similar amounts of [^{3}H]leucine-derived radiolabel. However, females on the low-protein diet (5 % protein) produced oothecae containing less [^{3}H]leucine-derived radiolabel. This reduction may reflect a general decline in radiolabel available for oogenesis in the amino acid pools of females placed on the 5 % protein diet 14 days before imaginal eclosion.

Fat-body urates appear to play an important role in cockroach nitrogen metabolism (Cochran, 1985; Mullins and Cochran, 1987; Wren and Cochran, 1987). Whole-body urate levels can be correlated with dietary nitrogen status and [^{14}C]urates are metabolized by cockroaches maintained on variable-nitrogen diets (Mullins and Cochran, 1987; Engebretson and Mullins, 1986). It also appears that oothecal urates are utilized during embryogenesis because their levels decline as the embryos develop (Mullins and Keil, 1980; Cochran and Mullins, 1982).

In this study, urate retention in females and its transfer to their oothecae was influenced by diet. It appears that females on the lower-nitrogen diet transfer more of their available urates into their oothecae. Females fed dog food retained more unlabelled and radiolabelled urate in their bodies than those on the 5 % protein diet. Oothecae from dog-food-fed females contained significantly more radiolabel (primarily as urates) than the female, but females on the 5 % protein diet contained significantly less ^{14}C radiolabel than their oothecae (Table 3).

Specific information on cockroach urate catabolism is not available (Wren and Cochran, 1987). However, significant quantities of $^{14}CO_2$ may be released in cockroaches provided with [^{14}C]urate stores (Cochran and Mullins, 1982; Engebretson and Mullins, 1986). The lower levels of [^{14}C]urate and total urate in the females fed on the 5 % protein diet can be attributed to increased metabolism of the stored urates, which is known to occur under circumstances of low dietary nitrogen (Table 4; Mullins and Cochran, 1987). Eighty-four per cent of the radiolabel contained in the oothecae obtained from dog-food-fed females and 75 % of the radiolabel contained in oothecae obtained from females fed on the 5 % protein diet was attributable to [^{14}C]urate. Because ^{14}C was largely retained as urates in the females, 98 % in all females, whether fed on dog food on the 5 %

protein diet, urates transferred to oothecae are metabolized to a greater extent during embryogenesis.

Radiotracer studies using [^{14}C]purine intermediates are useful only in studies of pathways involving carbon atoms but do not necessarily reflect the path of nitrogen contained in the urates. We conducted a preliminary experiment using a stable isotope of nitrogen ([^{15}N]urate) to determine whether ^{15}N could be incorporated into nitrogenous metabolites. We indentified four amino acids obtained from 21-day-old oothecae that contained measurable levels of ^{15}N enrichment. The ootheca obtained from a female maintained on the 5 % protein diet contained larger amounts of these ^{15}N-enriched amino acids than the ootheca from the female fed on dog food (Table 5). This information supports the hypothesis that urate-derived nitrogen is assimilated into nitrogen-containing compounds and that this mobilization is more pronounced in cockroaches on low-protein diets.

Female metabolism and utilization of urate metabolites or direct urate incorporation into oothecae is indicated by several lines of evidence. These include: (1) steadily decreasing urate levels during embryogenesis (Mullins and Keil, 1980) and fluctuations in birefringent 'white' granules, assumed to be urates, during various stages of embryogenesis (Tanaka, 1976); (2) the presence of ^{14}C radioactivity in tissue extracts that was not attributable to [^{14}C]urate (Mullins and Keil, 1980; Cochran and Mullins, 1982; this study), and (3) the finding that ^{15}N derived from [^{15}N]urate can be found in several amino acids (this study).

Much evidence has accumulated over the years implicating cockroach fat-body endosymbionts (bacteriocytes) in the metabolism of stored urates (Cochran, 1985; Wren and Cochran, 1987). These bacteriods are transmitted to oocytes during oogenesis (Sacchi *et al.* 1985) and are recognizable 10 days after the onset of embryogenesis (Tanaka, 1976). It is likely that they are involved in urate metabolism in the embryos. Nutritional symbiosis involving urate metabolism has been demonstrated in termites (Potrikus and Breznak, 1980*a*,*b*) and in molgulid tunicates (Saffo, 1988). In termites, uricolysis is anaerobic (Breznak, 1982). Wren and Cochran (1987) suggested that anaerobic uricolysis occurs in cockroach bacteriocytes.

Prezygotic paternal investment may include indirect nutritional contributions to progeny provided to females by males during mating (Zeh and Smith, 1985). Nutrient contributions may be derived from male accessory glands and/or emptied spermatophores consumed by females (Leopold, 1976; Thornhill, 1976; Markow and Ankney, 1984; Sakaluk, 1984). Zeh and Smith (1985) noted that physically harsh or biotically dangerous habitats and ephemeral, highly prized, productive resources may be associated with high levels of paternal investment. Some information is available among insects to support this hypothesis. In *Caryedon serratus* (Coleoptera; Bruchidae), male secretions may constitute an important trophic contribution that increases female fecundity and vitellogenesis (Boucher and Huignard, 1987). Interspecific mating differences between *Drosophila melanogaster* and *D. mojavensis* suggest that because *D. mojavensis* lives in a harsh

environment, often with limited resources, it remates more frequently in order to obtain nutrients from male ejaculates (Markow and Ankney, 1984). Cochran (1985) has suggested that, because of the geological period in which cockroaches evolved, the availability of dietary nitrogen was scarce or unpredictable. As a result, cockroaches may have been nitrogen scavengers. A portion of this strategy has been reviewed with respect to female urate (nitrogen) metabolism in a preceding section of this discussion. It appears that the males of many cockroach species may also be involved in nitrogen scavenging through uric acid found in association with the male accessory glands of some cockroach species (Roth and Dateo, 1964; Roth, 1967). Radiolabelled urates from male accessory gland urates are transferred to females in *B. germanica* (Mullins and Keil, 1980) and in *Xestoblatta hamata* (Schal and Bell, 1982). Schal and Bell (1982) found that female *X. hamata* feed on urates offered by males after copulation and that females on nitrogen-deficient diets ingest and transfer more male-derived urates to their maturing oocytes than do females on high-protein diets. *Blattella germanica* males provide more [^{14}C]urates to females on 5 % protein diets than to females on dog food, a major portion of which is then transferred to their oothecae (Mullins and Keil, 1980; Table 2, this study). Similarly, more [^{3}H]leucine-derived materials are transferred from the males to females with subsequent incorporation into their oothecae. The source of these [^{3}H]leucine-derived materials has not yet been established, but they are probably derived from spermatophores or seminal fluids. The nature of the materials contributed by males appears to be related to dietary nitrogen levels. Mated males fed on dog food provided about 13 % of their [^{3}H]leucine-derived radiolabel and 20 % of the [^{14}C]hypoxanthine-derived radiolabel (as urates) to females. In contrast, males fed on 5 % protein contributed 1 % of their [^{3}H]leucine-derived radiolabel and 41 % of their [^{14}C]hypoxanthine-derived radiolabel. Males on the low-protein diet apparently utilized the ^{3}H-labelled amino acid for their own short-term metabolic needs while providing more [^{14}C]urate to females. Females on the 5 % protein diet transferred ten times as much [^{14}C]urate into their oothecae as did those on dog food diets.

Several observations can be made in interpreting the results of the experiments in this study. First, the sizes of the metabolic pools in both male and female *B.germanica* are undoubtedly influenced by the diet to which they have access. Introduction of radiolabelled materials into metabolic pools of differing sizes will affect the specific activity of those materials in these pools. Thus, when the radiolabelled materials are utilized or transferred, the results obtained will reflect both the specific activity of these materials and the net amount of the materials transferred. Second, information provided in Fig. 1 can be used as an indicator of the significance of two radiolabelled resources injected into males which were then contributed by the males to the females and subsequently transferred by these females into their oothecae. These two nitrogen-containing materials are representative of a variety of compounds that could be transferred in a similar manner. The contribution obtained by females that is subsequently invested into their oothecae can be used as an indicator of the significance of paternal

investment. Finally, males can play a significant role in providing nutritional support to their progeny, particularly under circumstances where the female diet is nitrogen-deficient. This is supported by the finding that females on the 5 % protein diet incorporated more (63 %) of the total radiolabel made available to them by males than did those on the dog food diet (17 %) (Fig. 1).

We thank Drs D. G. Cochran, C. A. Nalepa and M. H. Ross for critical review of this manuscript. This research was supported in part by the Virginia Agricultural Experiment Station, Hatch project no. 130929.

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