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Expression, production and excretion of Bla g 1, a major human allergen, in relation to food intake in the German cockroach, *Blattella germanica*

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Abstract. The German cockroach, *Blattella germanica* (Linnaeus) (Dictyoptera: Blattellidae), produces several potent human allergens, one of which, *Blattella germanica* allergen 1 (Bla g 1), is produced in the midgut and excreted in faeces. We tested with descriptive and experimental approaches the hypothesis that Bla g 1 production is related to food intake in adult males and females of the German cockroach. Bla g 1 mRNA expression in the female midgut (assayed by real time quantitative polymerase chain reaction), her Bla g 1 content (assayed by enzyme-linked immunosorbent assay), and the female's faeces production and its Bla g 1 content tracked a cyclic pattern in relation to the gonadotrophic cycle. All four measures rose as food intake increased, declined before oviposition in relation to diminishing food intake, and remained low while the female carried an egg case for 20 days. After her first clutch of embryos hatched, the female resumed feeding, and faeces and Bla g 1 production increased concomitantly. Both Bla g 1 mRNA expression and Bla g 1 protein levels remained low in experimentally starved females. However, when starved females were allowed to feed, Bla g 1 production elevated and the gonadotrophic cycle resumed. Bla g 1 mRNA expression also increased six-fold in response to feeding compared to starved females. By contrast, there were no apparent cycles in the pattern of Bla g 1 production in males, reflecting their low and non-cyclic food intake. Our results therefore demonstrate that Bla g 1 production in *B. germanica* is modulated in relation to food intake.

Key words. Bla g 1, cockroach allergen, food intake, German cockroach.

Introduction

The German cockroach, *Blattella germanica* (Linnaeus), is an economically and medically important synanthropic pest, intimately associated with human-made structures (Schal & Hamilton, 1990; Brenner, 1995). This species has been recognized for several decades as a source of allergens, with a causative role in human allergic asthma (Kang, 1976; Kang *et al*., 1979). Sensitization and exposure to cockroach-produced allergens can lead to the development and onset of allergic respiratory disease – such as asthma – in atopic individuals, and is particularly important in 'inner-city', low-income homes where cockroach infestations can be very severe and persistent. Many studies have sought to develop approaches to mitigate the harmful effects of exposure to these indoor allergens. We recently showed, for example, that sustained reductions of *Blattella germanica* allergen 1 (Bla g 1, a major allergen produced by the German cockroach) below the human sensitization threshold for exposure (2 Units* Bla g 1/g of house dust; Eggleston *et al*., 1998) and the morbidity threshold (8 Units/g of house

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*Because there are no international reference standards for cockroach allergens, Bla g 1 content, as determined by ELISA, is expressed in arbitrary Units, per manufacturer calibration.

dust; Rosenstreich *et al*., 1997) could be achieved with effective pest control, sanitation, and resident education (Arbes *et al*., 2003, 2004).

Bla g 1, one of several known German cockroach allergens, has a variable molecular weight ranging from 33 to 37 kDa (Schou *et al*., 1990; Pollart *et al*., 1991). Bla g 1 shows 70–72% nucleotide sequence homology to the antigenically cross-reactive *Periplaneta americana* (American cockroach) allergen 1 (Per a 1), and together they comprise a family of structurally and antigenically related Group 1 allergens (Pomes *et al*., 1998; Melen *et al*., 1999). A unique feature of this group of cockroach allergens, and not previously found in other allergen sequences, is that they contain several tandem repeats of approximately 100 amino acids (Pomes *et al*., 1998). This protein also shows 94% sequence homology to a previously described 4-kb nucleotide sequence, Bla g Bd90K (Helm *et al*., 1996).

Recent immunochemical and molecular work has shown that not only is Bla g 1 found in overwhelmingly greater amounts in the midgut of the digestive system, but also is produced only by midgut cells (Gore & Schal, 2004). Furthermore, the production of Bla g 1 is neither sex- nor stage-specific, but adult females produce and excrete in their faeces significantly more Bla g 1 than do nymphs and adult males. In a 24-h period, a single adult female has the capacity to produce a level of Bla g 1 in her faeces that far exceeds the proposed human sensitization and morbidity thresholds for Bla g 1 exposure (Gore & Schal, 2004).

Although the function of Bla g 1 remains unknown, amino acid sequence analysis suggests a secretory pathway into the digestive system (Pomes *et al*., 1998). Bla g 1 shares 35–40% deduced amino acid sequence identity with AEG12 of *Aedes aegypti* (Accession No. AY038041) and ANG12 of *Anopheles gambiae* (Accession No. Q17040), both of which are bloodmeal-induced midgut proteins of the female mosquitoes (Müeller & Crisanti, unpublished, Accession No. Q17040; Morlais *et al*., 2003), suggesting a possible role in digestion. However, Bla g 1 also shares 37% amino acid sequence identity with the *Tenebrio molitor* cockroach allergen-like protein (Ferreira *et al*. unpublished, Accession No. AY327800), which like AEG12 (Shao & Jacobs-Lorena, unpublished; Accession No. AY050565) has been described as a microvillar membrane protein.

In this study, we investigate the relationship between Bla g 1 gene expression, its midgut and faeces contents, and patterns of food intake in adult male and female cockroaches. We also quantify Bla g 1 protein and mRNA expression over time in fed, starved, and re-fed adult females. Our results demonstrate that Bla g 1 production is modulated by feeding.

Materials and methods

Insects

Adult females were collected from a laboratory colony of insecticide-susceptible German cockroaches (American Cyanamid strain, Princeton, New Jersey, U.S.A.) reared in synchronous cohorts at 27 °C, under variable ambient relative humidity and a photoperiod of LD 12 : 12 h, and provided with water and rat chow (Purina no. 5012, Purina Mills, St. Louis, MO).

Bla g 1 extraction and quantitative enzyme-linked immunosorbent assay

Faeces or dissected tissues were homogenized in 1% bovine serum albumin–phosphate-buffered saline-Tween (1% BSA/PBS-T) containing protease inhibitors, using a hand-held pestle motor (Kimble/Kontes, Vineland, NJ, U.S.A.) and sterile disposable pestles (Kimble/Kontes). The homogenate was incubated with agitation on an orbital shaker at 4 °C for ~1 h, centrifuged at 8160 *g* for 10 min and the supernatants collected and stored at −80 °C until assayed. Bla g 1 titres were measured using a monoclonal capture and polyclonal detector enzyme-linked immunosorbent assay (ELISA) (Indoor Biotechnologies, Charlottesville, VA, U.S.A.) as described by Pollart *et al*. (1991) and Gore & Schal (2004).

Total RNA isolation and complementary DNA synthesis

Insects were cold-anaesthetized and dissected under cold cockroach saline. Total RNA was extracted from dissected tissues using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, U.S.A.) according to the manufacturer's instruction. Briefly, tissue was homogenized in 1 mL of TRIzol reagent. The homogenate was chloroform-extracted, precipitated with isopropyl alcohol, washed with 75% ethanol, and resuspended in RNase-free water. RNA samples were treated with RNase-free DNase (Ambion, Austin, TX, U.S.A.) to remove DNA contamination. The RNA concentration was determined spectrophotometrically at 260 nm. Aliquots of samples were stored at −80 °C until use. First strand cDNA was synthesized from 6 µg of total RNA using StrataScript reverse transcriptase (Stratagene, La Jolla, CA, U.S.A.) in the presence of oligo(dt) at 42 °C.

Real-time quantitative polymerase chain reaction

Primers and Taqman probes (Table 1) for Bla g 1 and a reference gene, *Blattella germanica* actin (Accession No. AY004248), were designed using the Assays-by-Design service (Applied Biosystems, Foster City, CA, U.S.A.). Polymerase chain reactions (PCR) were run in triplicate on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) in 25 µL containing 12.5 µL Taqman Universal PCR Master Mix (2 ×), 1.25 µL assay mix (20 ×), 6.25 µL RNase-free water and 5 µL diluted (0.2 ng/µL) cDNA. Amplification was performed under thermal cycling conditions as follows: 2 min at 50 °C, 10 min at 95 °C, and 40 cycles at 15 s at 95 °C and 1 min at 60 °C.

Table 1. Real time polymerase chain reaction primers and probes.

Name	Orientation	Oligonucleotide sequence 5′–3′	Nucleotide number
Bla g 1*	Forward primer	ACTAGTGAGAACTCAAGACTGATACGT	1235–1258
	Reverse primer	CGTGGAACAATCAAAATATAATACAAGTGTTCA	1350–1318
	Taqman probe	CCAAACTTCATTTCTGATAATAA	1269–1291
Actin†	Forward primer	GCATCACACCTTCTACAATGAACTC	90–114
	Reverse primer	CTGTTGGCCTTTGGGTTCAG	179–160
	Taqman probe	CCAGAGGAACACCCAATCC	124–142

*Bla g 1 primers and probes were designed based upon a non-repeating segment of the known cDNA sequence of clone Bla g 1.0101 (Accession No. AF072219).
†Actin primers and probe were designed based upon *Blattella germanica* actin (Accession No. AY004248).
Taqman probes were labelled with FAM at the 5′ end and TAMRA at the 3′ end.

Relative quantification of expression was determined using the comparative C_T method (relative quantification $= 2^{-\Delta\Delta C_T}$; Applied Biosystems User Bulletin #2: ABI PRISM 7700 Sequence Detection System). The threshold cycle (C_T) is the cycle number required for the reporter dye fluorescence to become greater than background fluorescence. Relative expression of target mRNA (Bla g 1) was determined relative to a calibrator (sample used for comparison; i.e. day 1 in Fig. 1B) and relative to the housekeeping gene ($\Delta\Delta C_T = \Delta C_T^{\text{sample}} - \Delta C_T^{\text{calibrator}}$, each $\Delta C_T = \Delta C_T^{\text{Bla g 1}} - \Delta C_T^{\text{actin}}$).

Age-related changes in Bla g 1 levels and expression in females and males

Levels of Bla g 1 protein were measured in adult females 1, 3, 5, 7, 9, 11 and 20 days after the imaginal moult ($n = 5$ per day). Females were mated on day 6 with 12-day-old adult males. Following homogenization and extraction of whole females, Bla g 1 was measured by ELISA.

To examine Bla g 1 levels in faeces, same-sex groups of 20 adult females ($n = 10$ groups) and males ($n = 10$ groups) were collected within 12 h of eclosion and placed in 13×18-cm plastic cages with food and water. Faeces was collected daily, weighed, extracted and Bla g 1 content measured by ELISA.

Temporal changes in expression of Bla g 1 mRNA were quantified. cDNA synthesized from total RNA of 1, 3, 5, 7, 9, 11, and 15-day-old adult females ($n = 3$ per day) was assayed using real-time quantitative PCR. Our previous results showed that Bla g 1 is produced exclusively in the midgut (Gore & Schal, 2004). Therefore, only the alimentary tract was used for quantitative expression analysis.

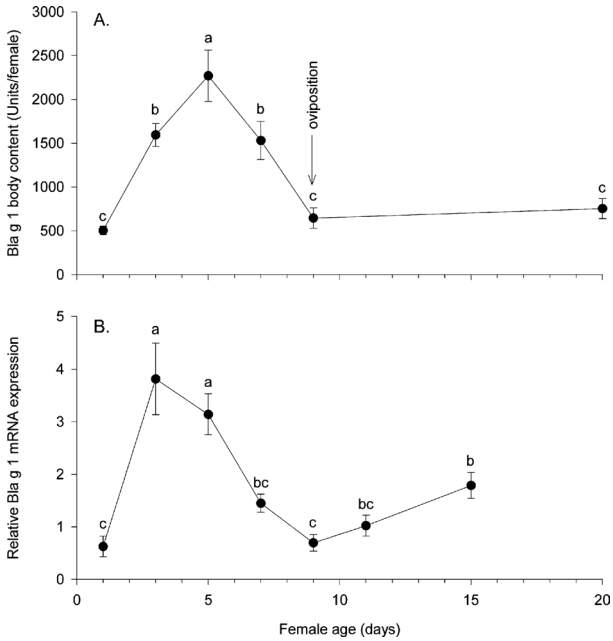

Fig. 1. (A) Changes in Bla g 1 content of adult female German cockroaches in relation to the reproductive cycle. Whole body Bla g 1 content was quantified by enzyme-linked immunosorbent assay and expressed as Units per female ± SEM ($n = 10$ females). (B) Relative changes in Bla g 1 mRNA expression of adult female German cockroaches in relation to the reproductive cycle. Values, representing the mean ± SEM ($n = 3$ alimentary tract cDNA per day), were normalized to *Blattella germanica* actin and day-1 values. Fold-change in expression was calculated using the formula: relative quantification $= 2^{-\Delta\Delta C_T}$. Means accompanied by different letters are significantly different (Fisher's LSD, $P < 0.05$).

Starvation effects on Bla g 1

The hypothesis that Bla g 1 protein levels change with levels of food intake was tested with starved adult female cockroaches. Adult females ($n = 5$) were collected within 12 h of eclosion, placed in 13×18-cm plastic cages and supplied with food and water for 2 days, followed by starvation through day 11. Another set of females also was starved on day 2, but food was returned on day 7 through day 11. Because cockroaches are coprophagous (Kopanic *et al.*, 2001), insects were moved to clean cages daily to minimize the ingestion of Bla g 1 contained in faeces. Whole alimentary canals, ligated at the anterior and posterior ends, were dissected and processed for assay by ELISA.

Differential expression of Bla g 1 mRNA in relation to feeding was also examined. cDNA synthesized from whole alimentary tract total RNA of day 11 females ($n = 3$ groups) from the previously described starvation regimes were assayed by real time quantitative PCR.

Statistical analyses

Whole body, gut and faeces (square root transformed) Bla g 1 contents, and the relative expression of Bla g 1 mRNA were subjected to analysis of variance (ANOVA; PROC GLM) in SAS 8.2 (SAS Institute, 2001). In pre-planned comparisons, means were compared using Fisher's least square difference (LSD; $\alpha = 0.05$).

Results

Bla g 1 protein and mRNA expression profiles of adult females

In the German cockroach, about 40 basal oocytes synchronously mature between days 0 and 9 of the first ovarian cycle (Schal *et al.*, 1997). Females oviposit on day 9, and although *B. germanica* is considered oviparous, females exhibit functional ovoviviparity: fertilized eggs are oviposited into an egg case (ootheca) that remains attached at the genital vestibulum of the female between days 9 and 28. During this 'pregnancy', embryogenesis proceeds and the new basal oocytes in the ovaries are prevented from growing. After hatch, the female resumes a new vitellogenic cycle.

The Bla g 1 content (quantified by ELISA and expressed in arbitrary Units) in adult females significantly changed ($F_{5,59} = 16.05$; $P < 0.0001$) in relation to the stage of their reproductive cycle. The Bla g 1 content increased during the vitellogenic stage (first 5 days) of the gonadotrophic cycle (Fig. 1A). After day 5, Bla g 1 levels significantly declined, and by oviposition on day 9 they reached a level not significantly different from that of day 1 females. Bla g 1 levels remained low through day 20, the middle of 'pregnancy'.

Bla g 1 mRNA expression also changed significantly over time ($F_{6,20} = 14.61$; $P < 0.0001$) in a similar cyclic pattern observed for the protein during the first half of the reproductive cycle (Fig. 1B). Relative to day 1, Bla g 1 mRNA was significantly up-regulated by day 3 (~four-fold) and day 5 (three-fold). Expression levels significantly declined after day 5 to a low on day 9. However, a significant increase in mRNA expression levels was evident by day 15, although only 1.7-fold higher than on day 1.

Faeces and its Bla g 1 content

Faeces production, an indirect measure of food intake, followed a distinct cyclic pattern in females, with a significant age effect ($F_{26,269} = 226.68$; $P < 0.0001$) (Fig. 2A). Following eclosion, the faeces mass per female per day significantly increased, peaked on day 4 (3.4 ± 0.2 mg), and subsequently declined ~54-fold by day 9. Faeces output remained low while the female carried an egg case (days 9–28). Following hatch on day 28, at the beginning of the second gonadotrophic cycle, faeces production immediately increased and followed a similar pattern as observed during the first cycle. Thus, faeces production followed the pattern of food intake in reproductive female cockroaches.

The Bla g 1 content of female faeces followed a similar cyclical pattern, but with a 2-day delay relative to faeces production (Figs. 2A and B). Faecal Bla g 1 per female increased in the first few days of the first gonadotrophic cycle, reaching a peak of 1188 ± 68 Units Bla g 1 on day 6, when mating occurred (Fig. 2A). Thereafter, Bla g 1 levels declined significantly (~12-fold), and remained low between oviposition on day 9 and hatching on day 28. The cycle of faecal output and faecal Bla g 1 repeated during the second gonadotrophic cycle between days 28 and 34 (Fig. 2A).

The concentration of Bla g 1 in faeces (Units/mg faeces) exhibited a similar cyclic pattern as described above (Fig. 2B). Interestingly, however, the highest Bla g 1 concentrations in female faeces appeared 2–5 days later than the peaks of faecal mass and Bla g 1 content in both the first and second gonadotrophic cycles. This suggests that faeces that is excreted late in each vitellogenic cycle contains more Bla g 1 per unit mass than faeces produced earlier and later in the gonadotrophic cycle.

Adult males produced approximately 10-fold less faeces than adult females (Fig. 3A). Moreover, unlike females, males showed no apparent cycles in either faecal mass per

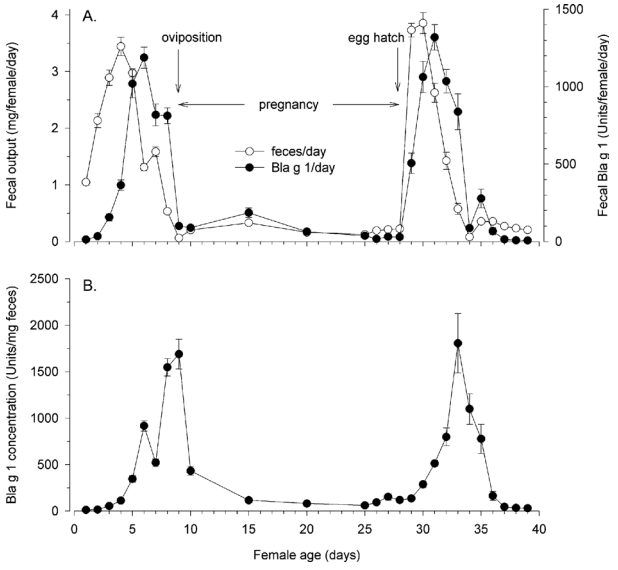

Fig. 2. (A) Changes in the mass of faeces excreted and faecal Bla g 1 content per adult female German cockroach per 24 h and (B) in the calculated concentration of Bla g 1 in faeces (Units per mg). Values represent the mean ± SEM ($n = 10$). Faecal Bla g 1 content was quantified by enzyme-linked immunosorbent assay and expressed as Units.

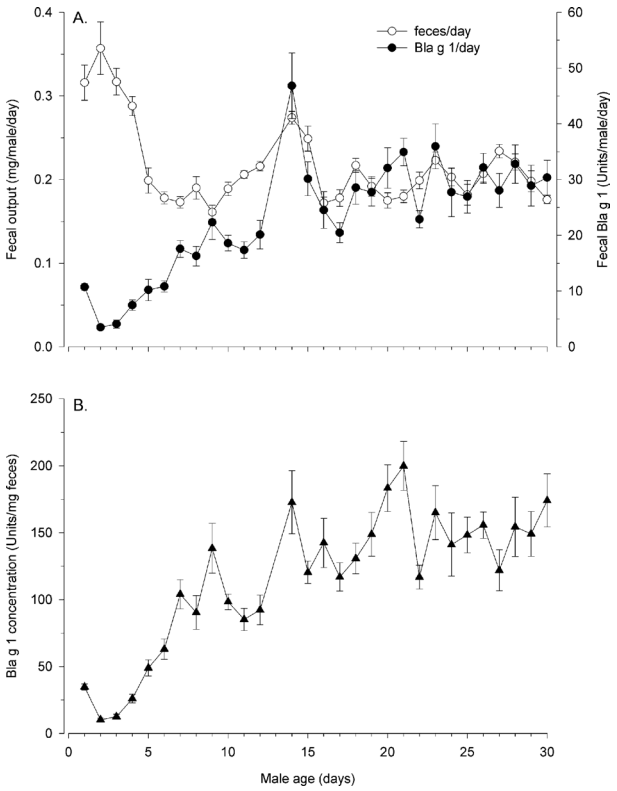

Fig. 3. (A) Changes in the mass of faeces excreted and faecal Bla g 1 content per adult male German cockroach per 24 h and (B) in the calculated concentration of Bla g 1 in faeces (Units per mg). Values represent the mean ± SEM ($n = 10$). Faecal Bla g 1 content was quantified by enzyme-linked immunosorbent assay and expressed as Units.

day or Bla g 1 content in faeces. Nevertheless, there was a significant age effect ($F_{28,279} = 15.35$; $P < 0.0001$), with more faeces produced by males during the first 4 days after adult eclosion and significantly less after day 5; faeces output after day 5 was also highly variable, and at times higher than in gravid females ($F_{1,19} = 68.37$; $P < 0.0001$). There also was a significant effect of age on faecal Bla g 1 levels per male ($F_{28,279} = 22.91$; $P < 0.0001$; Fig. 3A) and on Bla g 1 concentration ($F_{28,279} = 26.05$; $P < 0.0001$; Fig. 3B). However, unlike faeces mass, faecal Bla g 1 was lowest in the first 5 days, slowly increased during the next ~12 days, and fluctuated between ~20 and 35 Units Bla g 1 per male for the remainder of the assay period. The Bla g 1 concentration in male faeces (Units/mg faeces) peaked at a level 10-fold lower than in female faeces.

Effects of feeding and starvation on Bla g 1 protein and mRNA expression patterns

To examine the relationship between food intake and Bla g 1 production, adult females were starved after day 2 and changes in Bla g 1 content in dissected alimentary tracts were determined by ELISA. In fed females the pattern of Bla g 1 production in the alimentary tract was similar to that described for whole females, with highly significant modulation of Bla g 1 over time ($F_{8,44} = 12.30$; $P < 0.0001$; Fig. 4). Starvation after day 2 significantly affected the gut Bla g 1 content ($F_{2,109} = 8.94$; $P = 0.0003$; Fig. 4). In females fed only for the first 2 days after eclosion, Bla g 1 level increased two-fold by day 3 ($F_{8,44} = 5.12$; $P = 0.0003$). However, by day 5 their gut Bla g 1 content plateaued at a significantly lower level than in fed females (1608 ± 238 vs. 2703 ± 352, respectively; $F_{1,9} = 6.63$; $P = 0.033$). Bla g 1 levels remained unchanged for the remainder of the assay period in the absence of food.

The return of food to starved females on day 7 resulted in a significant stimulation of Bla g 1 production ($F_{8,44} = 15.06$; $P < 0.0001$; Fig. 4). After a delay of ~2 days, Bla g 1 significantly increased to 2664 ± 219 Units/gut by day 11, followed by an immediate decline. Interestingly, the decline in Bla g 1 between days 9 and 15 in re-fed females was not different from that observed between days 3 and 9 in continuously fed females. In an apparent return to normal physiological function, starved insects produced infertile oothecae after given access to food. It is noteworthy that on day 15, the Bla g 1 level of starved females was significantly greater ($F_{2,14} = 10.13$; $P = 0.003$) than in both sets of fed females that oviposited, suggesting that Bla g 1 oscillates between maximal and minimal levels under normal reproductive conditions, and remains intermediate in starved females.

The changes in Bla g 1 protein levels could be due to changes in mRNA expression or post-transcription events. Starved and re-fed day 11 females were assayed by real-time quantitative PCR and compared to day 11 fed control females. These treatments significantly altered the level of Bla g 1 mRNA expression in adult females ($F_{2,8} = 39.06$; $P = 0.002$). At this age, fed females had oviposited and exhibited low Bla g 1 mRNA expression (Figs. 1B and 5). When females were starved, Bla g 1 mRNA expression

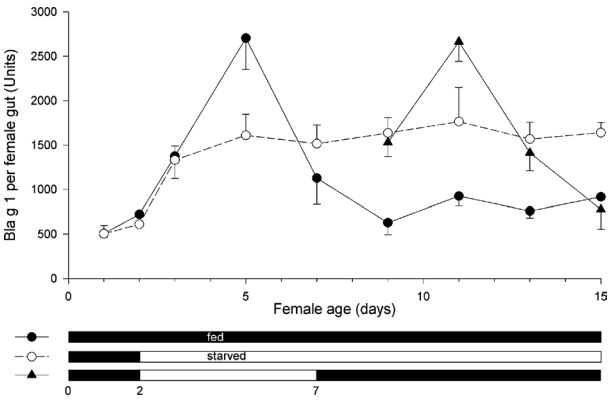

Fig. 4. Changes in gut Bla g 1 content in fed, starved and re-fed adult female German cockroaches. Starvation commenced on day 2, and some females were re-fed on day 7, as indicated by the schematic diagram below the abscissa (black bar = fed, grey bar = starved). Gut Bla g 1 content was quantified by enzyme-linked immunosorbent assay and expressed as Units per female gut ± SEM ($n = 5$).

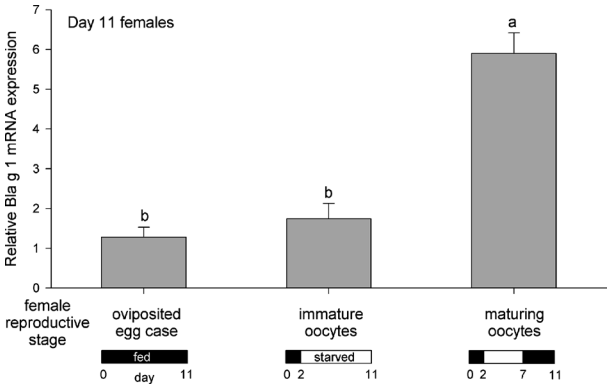

Fig. 5. Relative changes in Bla g 1 mRNA expression in 11-day-old fed, starved and re-fed adult female German cockroaches, treated as in Fig. 4. Values, representing the mean ± SEM ($n = 3$ alimentary tract cDNA), were normalized relative to *Blattella germanica* actin and day-11 fed females. Fold-change in expression was calculated using the formula: relative quantification $= 2^{-\Delta\Delta C_T}$. The reproductive stage of females on day 11 is represented on the abscissa, below which are schematic diagrams depicting the treatment (black bar = fed, grey bar = starved). Means with different letters are significantly different (Fisher's LSD, $P < 0.05$).

remained low. However, when food was returned to the insects after a 5-day starvation period, Bla g 1 expression was nearly six-fold higher, again confirming that food intake, or events associated with it, stimulate Bla g 1 production.

Discussion

This is the first report on the physiological modulation of the level of any of the six known German cockroach allergens, and indeed, to our knowledge, of any allergen of arthropod origin. The German cockroach has long been regarded as a pest of economic importance, and repeatedly implicated in the transmission of enteric disease. Since the first report ~40 years ago suggesting its involvement in human allergic response (Bernton & Brown, 1964), several potent allergens, including *Blattella germanica* allergen 1 (Bla g 1), have been identified and cloned from this cockroach (Schou *et al*., 1990; Stankus *et al*., 1990; Pollart *et al*., 1991; Arruda *et al*., 1995a, b, 1997; Chapman *et al*., 1998; Jeong *et al*., 2003). However, despite the plethora of information from clinical investigations on the impact of cockroach allergens on allergic individuals, and from environmental studies on the prevalence of allergens in infested structures, only recently has research begun to address some fundamental questions regarding the biology of the allergens produced by the German cockroach (Gore & Schal, 2004; Fan *et al*., 2005). The present study aimed to elucidate the hypothesized relationship between Bla g 1 production and events associated with reproduction, particularly in adult females of the German cockroach.

Quantitative analyses of Bla g 1 mRNA and production of the Bla g 1 protein in females indicated that both are closely modulated in relation to the reproductive cycle. Relative to day 1, Bla g 1 expression reaches maximal levels in vitellogenic females around days 3–5, followed by a dramatic decline through days 7–11, when the female completes the first oocyte maturation cycle and oviposits. The female's Bla g 1 protein content likewise increases during the first 5 days after adult eclosion, concomitantly with early stages of vitellogenesis, when the juvenile hormone titre increases and the basal oocytes mature (Schal *et al*., 1997). During pregnancy, a period marked by low juvenile hormone levels (Sevala *et al*., 1999; Cruz *et al*., 2003) the Bla g 1 titres remain low.

However, unlike juvenile hormone, which continues to rise through day 7 (Sevala *et al*., 1999), Bla g 1 levels decline considerably after day 5. It appears, therefore, that Bla g 1 production is less related to juvenile hormone and more so to food intake. Food consumption by adult female *B. germanica* follows a well-defined pattern. Food intake peaks around days 2–4, followed by a dramatic decline during which the female continues to provision her eggs, oviposits them into an egg case, and incubates her developing embryos for ~20 days. Only little and sporadic feeding occurs during this protracted 'pregnancy' (Lee & Wu, 1994; Schal *et al*., 1994; DeMark & Bennett, 1995; Osorio *et al*., 1998; Sevala *et al*., 1999).

The relationship between Bla g 1 and food intake was also explored experimentally by starving adult females. Whereas gut Bla g 1 levels of normally fed females exhibited a typical cycle that mirrored the gonadotrophic cycle, both starvation and re-feeding of starved females resulted in clear shifts in Bla g 1 production. In starved females we were able to halt the normal cyclic production of Bla g 1 at an intermediate level. When food was returned after 5 days of starvation, Bla g 1 production in the gut resumed at normal levels with a delayed peak and a subsequent decline, as in normally fed females. Quantitative analysis of mRNA expression levels confirmed the ELISA results, leading us to conclude that the production of Bla g 1 is up-regulated by food intake, or events associated with it.

These data, together with the results of Gore & Schal (2004) showing disproportionately higher Bla g 1 protein levels in the midgut than in the foregut and hindgut, raise at least two interesting possibilities about the physiological function(s) of Bla g 1. One hypothesis is that Bla g 1 may serve a digestive purpose. Bla g 1 shares 35–40% deduced amino acid sequence identity with ANG12 of *Anopheles gambiae* (Giles) (Accession No. Q17040) and AEG12 of *Aedes aegypti* (L.) (Accession No. AY038041), both of which are produced in the midgut of the female mosquito and undergo temporal changes in expression relative to the acquisition of a bloodmeal (Müeller & Crisanti, unpublished, Accession No. Q17040; Morlais *et al*., 2003). Furthermore, Bla g 1 sequences contain myristoylation and trypsin-cleavage sites, suggesting that it is post-translationally modified and secreted into the digestive tract.

Another interesting possibility is that Bla g 1 actually serves a structural rather than an enzymatic role in the midgut. Although Pomes *et al*. (1998), using sequence

analysis algorithms, suggested that Bla g 1 is not a membrane protein, but instead is secreted from the midgut epithelium through the rough endoplasmic reticulum, Bla g 1 also shares 37% amino acid sequence identity with the *Tenebrio molitor* cockroach allergen-like protein (Ferreira *et al*. unpublished, Accession No. AY327800), which, like AEG12 (Shao & Jacobs-Lorena, unpublished; Accession No. AY050565), has been described as a microvillar membrane protein. This presumed function might account for the observation that the Bla g 1 concentration in faeces is highest as feeding subsides. It suggests that microvilli might be sloughed off after large food boluses pass through the midgut. Further functional assays will be needed to resolve these two hypotheses.

Cockroach faeces is a source of several allergens (Richman *et al*., 1984; Zwick *et al*., 1991), and our recent results unambiguously showed that Bla g 1 is produced exclusively within the midgut and is excreted in faeces (Gore & Schal, 2004). Because faeces production is an indirect measure of food consumption, it is not surprising that the production of faeces, reported herein, follows very closely the patterns of food consumption previously reported for female *B. germanica*. Adult males have no discrete feeding patterns and eat comparatively less than females (Hamilton & Schal, 1988; DeMark & Bennett, 1995), a point reflected in our results, which show that, in general, females produce nearly an order of magnitude more faeces than males, especially during the vitellogenic stage.

In general, the Bla g 1 content of female faeces follows the patterns of faeces production. Surprisingly, however, greater faecal mass does not always correspond to higher Bla g 1 levels. Rather, faecal Bla g 1 tends to peak 1–2 days after maximal faeces excretion, possibly related to a delay in the up-regulation of the Bla g 1 transcript relative to food intake (see Fig. 1). This delay is most apparent near oviposition, on days 8 and 9 of the first gonadotrophic cycle, and again on day 33 of the second cycle, when faeces production is minimal but the concentration of Bla g 1 in female faeces peaks at >1700 Units/mg faeces. These high concentrations in females represent $\sim$12-fold more Bla g 1 per mg faeces than in males.

Females consistently produced more Bla g 1 than males, as previously noted for vitellogenic females (Gore & Schal, 2004). Following oviposition, however, faecal Bla g 1 dramatically declined and remained low during pregnancy, a protracted period during which Bla g 1 in female faeces was typically lower than in males, albeit only slightly so. Nonetheless, over the course of a single reproductive cycle ($\sim$28 days), females excrete $\sim$three-fold more faeces and produce $\sim$eight-fold more Bla g 1 than males. Moreover, as adult females tend to live nearly twice as long as males (Ross & Mullins, 1995), the potential lifetime input of a single adult female to environmental Bla g 1, from faeces alone, would be $\sim$25 000–50 000 Units compared to $\sim$2000–3000 Units for males. However, the tendency of males to travel greater distances than females (DeMark & Bennett, 1995) may result in spreading of their allergen-laden faeces over a larger residential area. In any case, it is still apparent that both sexes are quite capable of excreting enough allergen to easily surpass the human sensitization (2 Units/g of environmental dust) (Eggleston *et al*., 1998) and morbidity (8 Units/g of environmental dust) (Rosenstreich *et al*., 1997) thresholds for exposure, even during periods of low faeces excretion.

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