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Expressed sequence tags analysis of *Blattella germanica*

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Abstract: Four hundred and sixty five randomly selected clones from a cDNA library of *Blattella germanica* were partially sequenced and searched using BLAST as a means of analyzing the transcribed sequences of its genome. A total of 363 expressed sequence tags (ESTs) were generated from 465 clones after editing and trimming the vector and ambiguous sequences. About 42% (154/363) of these clones showed significant homology with other data base registered genes. These new *B. germanica* genes constituted a broad range of transcripts distributed among ribosomal proteins, energy metabolism, allergens, proteases, protease inhibitors, enzymes, translation, cell signaling pathways, and proteins of unknown function. Eighty clones were not well-matched by database searches, and these represent new *B. germanica*-specific ESTs. Some genes which drew our attention are discussed. The information obtained increases our understanding of the *B. germanica* genome.

Key words: *Blattella germanica*, cDNA library, ESTs, BLAST search, novel genes

INTRODUCTION

The cockroach is among the oldest winged insects known, and its habits are closely associated with those of humans. Over four thousand species of cockroach are known, and about thirty species are harmful to humans in various ways. The importance of the German cockroach has been emphasized because it is the most populous and has the widest distribution (Ross and Cochran, 1975). *Blattella germanica* is also a well known cause of allergic diseases, rather than acting as a vector of infectious diseases (Richman et al., 1984).

Previous genetic studies on *B. germanica* have been limited to the study for some of its genes, e.g., allergen genes (Arruda et al 1995; Helm et al., 1996) and the cytochrome P450 gene, which is related with juvenile hormone or insecticide tolerance (Martinez-Gonzalez and Hegardt 1994; Scharf et al., 1998).

The generation and analysis of expressed sequence tags (ESTs) provides useful information on development, metabolism, virulence factors, drug targets, and pathogenesis in various organisms (el-Sayed et al., 1995; Wu et al., 1996; Manger et al., 1998; Bahl et al., 2003).

To understand more about the expression pattern of its genome, we generated ESTs from a cDNA library of *B. germanica*. The analysis of such data provides valuable insights into the metabolism and growth of German cockroach.

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MATERIALS AND METHODS

Cockroach breeding

Adult male and female German cockroaches were reared at 24°C on an artificial diet, and given free access to water

cDNA library construction

A *B. germanica* cDNA library was constructed using Uni-ZAP^{TM}-XR expression vector (Stratagene, USA). In brief, total RNA was isolated from 3 g of the midgut of adult *B. germanica*. After phenol extraction and ethanol precipitation, poly(A^+) RNA was purified using a Stratagene Poly(A) Quick mRNA Isolation Kit, in accordance with the manufacturer's instructions. First-strand cDNA synthesis of the isolated poly(A^+) RNA was then conducted in 50 μl reaction volumes, using 50 units of MMLV-reverse transcriptase at 37°C for 60 minutes. cDNA synthesis was primed using 5 μg oligo dT18. Second-strand synthesis was then conducted using RNase H and DNA polymerase I. After blunting the cDNA termini, *Eco*R I adaptor ligation and *Eco*R I phosphorylation were performed. Gel regions containing DNA molecules of length <400bp were then removed by Sepharose CL-2B gel filtration. Purified cDNA was ligated using dephosphorylated *Eco*R I Uni-ZAP^{TM}-XR vector arms, according to the manufacturer's instructions (Stratagene, USA), and then incubated using in vitro packaging extracts (Stratagene, USA).

Sequencing of randomly selected cDNA clones

Colonies from *E. coli* XL-1 Blue MRF cells harboring plasmid were obtained en masse by in vivo excision using assistant helper phage. Random recombinant clones were selected by blue-white color selection of colonies grown on LB-ampicillin agar plates. Plasmids containing the cDNA insert were extracted using a Wizard plasmid DNA purification system (Promega, Madison, WI, USA), and the existence of cDNA inserts was confirmed by gel electrophoresis after double digestion with *Eco*R I and *Xho* I. cDNA inserts were sequenced at DNA Sequencing Service (Macrogen, Seoul, Korea).

Basic local alignment search tool (BLAST) search analysis

Sequence outputs were manually edited to remove vector and ambiguous sequences. Sequence outputs of <100 bases in length were also rejected. The sequence data of cDNA clones obtained by random partial sequencing were searched for using BLAST at the National Center for Biotechnology Information (NCBI) for similarities in nucleic acid and protein databases. The BLASTN algorithm was used in conjunction with a nucleotide sequence database with a probability (P) cut-off of 10^{-4}. Matches of translational products versus nucleic acid sequences search for using the BLASTX algorithm with a probability (P) cut-off of 10^{-4}. Scores >160 for BLASTN or >80 for BLASTX were considered significant.

RESULTS

The submitted ESTs for BLAST searching comprised 363 ESTs from 465 randomly selected clones of the cDNA library of *B. germanica*. Clones of <100 bp in length or not successfully sequenced were excluded (102 clones). The average size of the 363 ESTs was 604 bp. These 363 sequenced clones were divided into three groups based on matches with public data sequences (Table 1). The matched with database group of 154 clones, showed high homology with the DNA sequence of *B. germanica* or other organisms. Of these clones, 10 ESTs corresponded to 3 previously identified *B. germanica* genes, including cytochrome c

Table 1. Composition and ESTs categories of *Blattella germanica* cDNA library

Group	No. of clones
Total clones sequenced	465
ESTs submitted to dbEST database	363
Match to database	154
Clone with homology to *B. germanica* (redundant clones 7)	10
Clone with homology to other organisms (redundant clones 34)	144
Non-match to database	80
Non-significant clones	129

Table 2. Database match of *Blattella germanica* EST to the genes of the other organisms

Clone No	Length	Accession No	Putative homologue	Organism	Score	P(N)
Ribosomal protein						
Bg11019	831	NP_524726	ribosomal protein L8	*Drosophila melanogaster*	83	4.00E-15
Bg9035	659	AAL26575	ribosomal protein L8	*Spodoptera frugiperda*	311	3.00E-84
Bg9065	644	AAK76989	ribosomal protein L9	*Spodoptera frugiperda*	327	4.00E-89
Bg3103	381	AAK83857	ribosomal protein L17/23	*Spodoptera frugiperda*	154	2.00E-37
Bg10031	863	AAL62470	ribosomal protein L18A	*Spodoptera frugiperda*	259	3.00E-68
Bg9046	286	AAL26577	ribosomal protein L29	*Spodoptera frugiperda*	108	1.00E-23
Bg9031	443	CAC19413	ribosomal protein L31	*Heliothis virescens*	186	5.00E-47
Bg11023	504	AAK92169	ribosomal protein L35A	*Spodoptera frugiperda*	163	8.00E-40
Bg9025	354	AAK92172	ribosomal protein L37A	*Spodoptera frugiperda*	155	8.00E-38
Bg12004	520	NP_476874	ribosomal protein S2	*Drosophila melanogaster*	226	1.00E-58
Bg11037	829	AAL26579	ribosomal protein S3A	*Spodoptera frugiperda*	348	5.00E-95
Bg9014	562	NP_524884	ribosomal protein S14	*Drosophila melanogaster*	212	2.00E-54
Bg11059	951	AAK92190	ribosomal protein S21	*Spodoptera frugiperda*	57	2.00E-07
Bg7009	357	P47991	60S ribosomal protein L6	*Caenorhabditis elegans*	73	1.00E-13
Bg9060	295	P32429	60S ribosomal protein L7A	*Gallus gallus*	75	2.00E-13
Bg8043	937	O96647	60S ribosomal protein L10	*Bombyx mandarina*	131	2.00E-31
Bg4022	563	P46222	60S ribosomal protein L11	*Drosophila melanogaster*	303	2.00E-82
Bg8048	407	P41126	60S ribosomal protein L13	*Drosophila melanogaster*	119	1.00E-27
Bg8031	619	P41093	60S ribosomal protein L18A	*Drosophila melanogaster*	249	7.00E-72
Bg6016	832	P36241	60S ribosomal protein L19	*Drosophila melanogaster*	164	2.00E-40
Bg4018	504	P23131	60S ribosomal protein L23	*Homo Sapiens*	215	3.00E-56
Bg8025	550	Q02877	60S ribosomal protein L26	*Homo Sapiens*	133	2.00E-31
Bg7021	505	P46615	60S ribosomal protein L32	*Drosophila pseudoobscura*	234	7.00E-62
Bg7041	514	P02433	60S ribosomal protein L32	*Homo Sapiens*	167	8.00E-42
Bg9006	888	AAK921	60S ribosomal protein L35	*Spodoptera frugiperda*	114	1.00E-24
Bg8001	352	Q962S7	60S ribosomal protein L37	*Spodoptera frugiperda*	114	4.00E-26
Bg8015	572	P05389	60S acidic ribosomal protein P2	*Drosophila melanogaster*	94	1.00E-19
Bg5006	819	P52813	40S ribosomal protein S3A	*Anopheles gambiae*	268	7.00E-81
Bg9071	221	P55830	40S ribosomal protein S3A	*Drosophila melanogaster*	64	3.00E-10
Bg10029	947	P02350	40S ribosomal protein S3A	*Xenopus laevis*	147	3.00E-36
Bg11001	890	P47835	40S ribosomal protein S3B	*Xenopus laevis*	285	7.00E-79
Energy metabolism						
Bg7011	871	P33502	NADH-Ubiquinone oxidoreductase chain 1	*Anopheles quadrimaculatus*	273	4.00E-73
Bg8035	848	P29867	NADH-Ubiquinone oxidoreductase chain 2	*Drosophila mauritiana*	119	6.00E-27
Bg9045	899	Q34048	NADH-Ubiquinone oxidoreductase chain 4	*Ceratitis capitata*	248	5.00E-65
Bg7025	510	Q34050	NADH-Ubiquinone oxidoreductase chain 6	*Ceratitis capitata*	112	4.00E-25
Bg1003	564	P07704	cytochrome b	*Drosophila yakuba*	241	4.00E-64
Bg9056	836	AAG17094	cytochrome b	*Bifiditermes improbus*	217	2.00E-81
Bg10006	942	AAG17097	cytochrome b	*Cryptotermes cynocephalus*	230	1.00E-59
Bg6013	905	P00400	cytochrome c oxidase polypeptide I	*Drosophila yakuba*	207	1.00E-53
Bg7003	219	P00399	cytochrome c oxidase polypeptide I	*Drosophila melanogaster*	51	5.00E-07
Bg8054	123	P50671	cytochrome c oxidase polypeptide I	*Choristoneura rosaceana*	42	2.00E-04
Bg5016	695	P29877	cytochrome c oxidase polypeptide II	*Periplaneta americana*	269	3.00E-72

Table 2. continued

Clone No	Length	Accession No	Putative homologue	Organism	Score	P(N)
Bg8064	150	P98048	cytochrome c oxidase polypeptide II	*Yponomeuta malinellus*	62	2.00E-10
Bg8068	379	P29877	cytochrome c oxidase polypeptide II	*American cockroach*	173	5.00E-44
Bg4013	783	P14574	cytochrome c oxidase polypeptide III	*Locusta migratoria*	223	4.00E-58
Bg8045	883	P00417	cytochrome c oxidase polypeptide III	*Drosophila melanogaster*	221	1.00E-57
Bg12010	983	AAB31450	cytochrome c oxidase subunit I	*Blattella germanica*	203	3.00E-51
Bg3202	387	AAF89137	cytochrome oxidase subunit III	*Cicindela belfragei*	152	9.00E-37
Bg10013	740	AAG01168	cytochrome oxidase subunit III	*Samia cynthia ricini*	229	2.00E-59
Bg3209	430	BAA32127	cytochrome oxidase II	*Blattella germanica*	244	9.00E-65
Bg7024	795	Q9V4U9	cytochrome P450 6a13	*Drosophila melanogaster*	120	3.00E-27
Allergen						
Bg8050	287	AAB82404	Cr-PII	*Periplaneta americana*	58	2.00E-08
Bg10001	294	AAC34737	Cr-PII allergen	*Periplaneta americana*	58	2.00E-08
Bg1010	340	AAD13530	major allergen Blag1.0101	*Blattella germanica*	121	2.00E-27
Bg7008	365	AAD13532	major allergen Blag1.0101	*Blattella germanica*	135	1.00E-31
Protease						
Bg6009	408	P35035	Trypsin 1 precursor	*Anopheles gambiae*	123	1.00E-28
Bg3106	241	P35036	Trypsin 2 precursor	*Anopheles gambiae*	87	3.00E-17
Bg4101	439	S35339	trypsin (EC 3.4.21.4) 1 precursor	*Anopheles gambiae*	123	4.00E-28
Bg11002	384	AAD31269	trypsinogen Rdo T3 precursor	*Rhyzopertha dominica*	130	2.00E-30
Bg3109	382	P04069	Carboxypeptidase B	*Astacus astacus*	84	3.00E-16
Bg11072	430	1EQ9A	Chain A, Crystal Structure Of Fire Ant Chymotrypsin	*Solenopsis invicta*	86	7.00E-17
Bg11049	534	AAA97479	Astryp1	*Anopheles stephensi*	129	2.00E-29
Enzyme related to metabolism						
Bg4008	607	Q9Y600	Cysteine sulfinic acid decarboxylase	*Homo sapiens*	57	3.00E-08
Bg6014	838	Q59296	Catalase	*Campylobacter jejuni*	67	3.00E-11
Bg8004	757	P26221	Endoglucanase E-4 precursor	*Thermobi fidafusca*	134	1.00E-31
Bg9010	847	S41881	alpha-amylase (EC 3.2.1.1) 1 precursor	*Litopenaeus vannamei*	192	4.00E-48
Bg9051	607	BAB91145	beta-glucosidase	*Neotermes koshunensis*	82	3.00E-15
Bg6015	442	P49010	beta-N-acetylglucosaminidase precursor	*Bombyx mori*	120	8.00E-28
Bg9015	949	P18173	Glucosedehydrogenase	*Drosophila melanogaster*	100	2.00E-20
Bg9043	589	JC4081	surcease/fructanase precursor	*Actinomyces naeslundii*	52	3.00E-06
Bg11053	924	AAC79122	alpha-amylase	*Drosophila ananassae*	195	6.00E-49
Bg10043	869	A34406	aldehydereductase (EC 1.1.1.21)	*Oryctolagus cuniculus*	122	6.00E-27
Bg9033	835	AAB61345	lysozyme	*Anopheles darlingi*	69	6.00E-11
Bg10002	453	BAB33297	Esterase-like protein (ESR-LP)	*Bombyx mori*	67	8.00E-11
Protease inhibitor						
Bg5047	340	Q06684	Rhodniin (Thrombin inhibitor)	*Rhodnius prolixus*	58	3.00E-09
Bg9028	404	S45677	proteinase inhibitor	*Pacifastacus leniusculus*	44	4.00E-04
Bg10033	1026	AAK57342	thrombin inhibitor infestin precursor	*Triatoma infestans*	64	2.00E-09
Translation						
Bg9029	888	NP_524611	elongation factor 1 alpha 100E	*Drosophila melanogaster*	233	3.00E-60
Bg9007	377	P29522	elongation factor 1-β'	*Bombyx mori*	66	5.00E-41
Bg12018	758	BAB21109	elongation factor 1 delta	*Bombyx mori*	130	1.00E-29
Bg5012	486	Q9VL18	elongation factor 1-delta	*Drosophila melanogaster*	67	2.00E-11

Table 2. continued

Clone No	Length	Accession No	Putative homologue	Organism	Score	P(N)
Cell signaling pathway						
Bg11067	384	Q09966	Putative G protein-coupled receptor B0244.7	*Caenorhabditis elegans*	30	3.9
Others						
Bg8028	901	P14792	Ubiquitin	*Caenorhabditis elegans*	109	5.00E-14
Bg10044	322	NP_476776	Ubiquitin fusion 52	*Drosophila melanogaster*	140	3.00E-33
Bg5014	484	P22943	12kDa heat shock protein	*Saccharomy cerevisiae*	94	1.00E-19
Bg7040	829	P41822	ferritin subunit precursor	*Aedes aegypti*	82	8.00E-16
Bg9050	583	NP_523683	Peroxiredoxin2540	*Drosophila melanogaster*	84	2.00E-19
Bg7005	492	O43653	Prostate stem cell antigen precursor	*Homo Sapiens*	42	5.00E-04
Bg4010	359	P40618	High mobility group protein 4 (HMG-4)	*Gallus gallus*	55	3.00E-08
Bg3208	433	AAH10444	matrilin2	*Homo Sapiens*	49	1.00E-05
Bg8012	395	AAM21357	mucin-like protein 1	*Ctenocephalides felis*	49	8.00E-06
Bg8016	842	O76767	ER lumen protein retaining receptor	*Drosophila melanogaster*	140	3.00E-33
Bg8039	447	Q27377	odorant-binding protein A10 precursor	*Drosophila melanogaster*	5	1.00E-06
Bg9016	880	AAA51540	4F2 antigen heavy chain	*Homo sapiens*	71	1.00E-11
Not classified						
Bg9041	645	AAF45949	CG3556 gene product	*Drosophila melanogaster*	85	5.00E-16
Bg9040	401	NP_611703	CG4250 gene product	*Drosophila melanogaster*	63	7.00E-10
Bg11018	927	AAF55754	CG4362 gene product	*Drosophila melanogaster*	84	3.00E-15
Bg9020	902	NP_611243	CG6459 gene product	*Drosophila melanogaster*	133	3.00E-30
Bg9037	304	AAF50709	CG6592 gene product	*Drosophila melanogaster*	65	1.00E-10
Bg10012	854	NP_612081	CG9119 gene product	*Drosophila melanogaster*	90	2.00E-19
Bg9069	425	AAF48872	CG6696 gene product	*Drosophila melanogaster*	77	3.00E-14
Bg9044	833	AAF56428	CG10423 gene product	*Drosophila melanogaster*	118	9.00E-26
Bg7001	899	AAF58797	CG12405 gene product	*Drosophila melanogaster*	100	3.00E-20
Bg8018	285	P30652	23.7KD protein ZK6436 in chromosome III	*Caenorhabditis elegans*	51	4.00E-07
Bg9003	484	A45835	Ly6 homolog RK10 precursor	*Norway rat*	47	8.00E-05
Bg9055	877	AAF91388	SocE	*Myxococcus xanthus*	99	4.00E-20
Bg11070	856	NP_523610	clumsy	*Drosophila melanogaster*	123	2.00E-34
Bg11054	864	NP_476631	RpL19-P1;Enhancer of Delta KP135	*Drosophila melanogaster*	143	2.00E-33
Bg12020	226	E81737	hypothetical protein TC0128	*Chlamydia muridarum*	44	4.00E-04
Bg4020	841	P34472	136.3kD a protein F58A4.5 in chromosome III	*Caenorhabditis elegans*	76	6.00E-14
Bg9061	245	AAL49280	RE74144p	*Drosophila melanogaster*	44	5.00E-04
Bg10005	634	NP_502360	Arabidopsis pathogenesis-related protein 5 like	*Caenorhabditis elegans*	109	2.00E-23
Bg11011	547	AAL31950	CDH1-D	*Gallus gallus*	51	7.00E-14

oxidase subunit I, cytochrome oxidase II, and major allergen Bla g 1. The non-matched to database group contained 80 clones. One hundred twenty nine clones weren't significant.

ESTs were classified into putative function categories based on BLAST search results with associated predicted or known functions (Table 2). The most frequently found gene was that of ribosomal protein as 31 clones (27%). Nineteen genes (18%) were of uncertain function. Twenty, 12 and 12 ESTs corresponded

to energy metabolism, enzymes related to metabolism and others, and 4 ESTs to allergens, 7 to proteases, 3 to protease inhibitors, 4 to translation factors, and 1 to cell signaling pathway.

DISCUSSION

The ESTs of 363 clones from a randomly selected 464 clones of *B. germanica* cDNA library were submitted for BLAST search and analyzed to determine the transcribed genome sequences. One hundred fifty four matched ESTs showed high homology with genes of various organisms including *B. germanica*. The ESTs of 41 clones showed the redundancy to other ESTs. Ten clones that had shared exact homology with genes previously characterized in *B. germanica* corresponded to 3 genes, cytochrome oxidase subunit 1, cytochrome oxidase II, and major allergen Bla g 1. Sixty-five ESTs were confirmed from among the 80 not-matched clones.

The most abundant group of ESTs in this study belonged to the ribosomal proteins. This result was expected because ribosomal protein genes are expressed ubiquitously at all stages of development. Moreover, the ribosomal protein family is generally well conserved and contains about 55 proteins in prokaryotes and 88 in eukaryotes (Doudna and Rath, 2002). An increasing number of studies have reported that numerous ribosomal proteins have extra-ribosomal functions, such as, involvements with several human genetic disorders (Wool, 1996). A recent study reported that ribosomal protein promotes DNA base excision repair in mammals such as the human and the mouse. This protein gene was expected to be used to repair 8-oxoguanine in man (Cappelli et al., 2003). In addition, the ribosomal protein family provides valuable comparative genomic and phylogenic data on insecta (Landais et al., 2003).

Cytochrome ESTs followed ribosomal proteins in number. These included cytochrome b, cytochrome oxidase polypeptides I, II, and III, cytochrome oxidase subunits II, and III, and cytochrome P450. These abundances could be explained by the high mRNA expression levels of cytochrome c oxidase subunit 1 in gut and fat bodies (Martinez-Gonzalez and Hegardt, 1994). The cytochrome c oxidase subunit I and the cytochrome P450 genes are over-expressed in pyrethroid-resistant strains of *B. germanica* (Pridgeon and Liu, 2003). Moreover, the cytochrome oxidase and P450 genes are good targets for the control of insecticide resistant German cockroach. The complete nucleotide sequences of the mitochondrial genome of several insects were recently identified for several purposes, such as, medicinal, sanitational, and forensic (Bae et al., 2004; Kim et al., 2005). The complete cockroach mitochondrial genome will be a useful source of information for molecular and evolutionary studies and for cockroach control.

B. germanica have been reported to have n=11 or n=12 chromosomes (Cochran and Ross, 1967; Ock and Kim, 1989). Although no specific information is available on the genome size of the German cockroach, it has been estimated to be ca. 1×10^{10} bp and CV=2.0 (haploid c-value in pg) (Ussery and Hallin, 2004), which is three times as large as the human genome. Wen et al (2001) reported that the *B. germanica* P450 gene is related to five pseudogenes compared to two pseudogenes in *Drosophila*. These pseudogenes, especially nuclear mitochondrial pseudogenes, have recently been viewed as tools for clarifying the relationship between DNA loss and genome size. Bensasson et al., (2001) reported that rates of DNA loss in pseudogenes are slow in the mountain grasshopper. However, in *Drosophila*, rates were high enough to contribute to the paucity of pseudogene sequences in the genome. The presence of many copies of pseudogenes is likely to explain the large genome size of *B. germanica*.

Of the protease genes identified in this study, trypsin influences growth and metamorphosis. Aalberse (2000) classified about 40 allergens into 4 structural families and other structures and designated trypsin-like serine proteases as one group of the antiparallel β-strands family. Moreover, there are reports that proteases extracted from *B. germanica* may have allergenic properties (Iraneta et al., 1999; Wongtim et al., 1993). We confirmed in a previous study that the trypsin of *B. germanica* reacts with the

sera of allergic patients (Ock et al., 2005). A further characterization of trypsin in this respect would provide information of allergy, since trypsin plays an important part in the activation of PAR-2 (protease-activated receptor-2).

The clone Bg9033 was identified as a lysozyme. The secretion of lysozymes is known to be increased in the gastrointestinal tract of *B. germanica* during metamorphosis and food ingestion (Aigaki et al., 2002). Thus genetic information on lysosome would be helpful in studies of cockroach metamorphosis and digestion. The clone Bg6014, a catalase is known to affect defense mechanism and to expand life span by blocking free hydroxyl radical production in *Drosophila* (Hotokezaka et al., 2002; Missirlis et al., 2001). Further studies on catalase, cytochrome oxidase, and P450 would provide information useful for cockroach control.

In addition, elongation factors, ubiquitin, iron storage protein ferritin, and G protein-coupled receptor were all confirmed to be present in the German cockroach.

In the present study, we found 363 cDNA clones in the German cockroach genome, and 360 of these were identified for the first time in the German cockroach. These ESTs should provide valuable information on the development and metabolism of *B. germanica* and lead to the discovery of control targets.

REFERENCES

Aalberse RC (2000) Structural biology of allergens. *J Allergy Clin Immunol* **106:** 228-238.

Aigaki T, Seong KH, Matsuo T (2002) Longevity determination genes in *Drosophila melanogaster*. *Mech Ageing Dev* **123:** 1531-1541.

Arruda LK, Vailes LD, Mann BJ, Shannon J, Fox JW, Vedvick TS, Hayden ML, Chapman MD (1995) Molecular cloning of a major cockroach (*Blattella germanica*) allergen, Bla g 2. *J Biol Chem* **270:** 19563-19568.

Bae JS, Kim I, Sohn HD, Jin BR (2004) The mitochondrial genome of the firefly, *Pyrocoelia rufa*: complete DNA sequence, genome organization, and phylogenetic analysis with other insects. *Mol Phylogenet Evol* **32:** 978-85.

Bahl A, Brunk B, Crabtree J, Fraunholz MJ, Gajria B, Grant GR, Ginsburg H, Gupta D, Kissinger JC, Labo P, Li L, Mailman MD, Milgram AJ, Pearson DS, Roos DS, Schug J, Stoeckert CJ Jr, Whetzel P. Plasmo DB (2003) the *Plasmodium* genome resource. A database integrating experimental and computational data. *Nucleic Acids Res* **31:** 212-215.

Bensasson D, Petrov DA, Zhang DX, Hartl DL, Hewitt GM (2001) Genomic gigantism: DNA loss is slow in mountain grasshoppers. *Mol Biol Evol* **18:** 246-253.

Cappelli E, D'Osualdo A, Bogliolo M, Kelley MR, Frosina G (2003) *Drosophila* S3 ribosomal protein accelerates repair of 8-oxoguanine performed by human and mouse cell extracts. *Environ Mol Mutagen* **42:** 50-58.

Cochran DG, Ross MH (1967) Preliminary studies of chromosome of twelve cockroach species (Blattaria: Blattidae, Blattallidae, Blaberidae). *Ann Ent Soc Amer* **60:** 1265-1267.

Doudna JA, Rath VL (2002) Structure and function of the eukaryotic ribosome; the next frontier. *Cell* **109:** 153-156.

el-Sayed NM, Alarcon CM, Beck JC, Sheffield VC, Donelson JE (1995) DNA expressed sequence tags of *Trypanosoma burcei rhodesiense* provide new insights into the biology of the parasite. *Mol Biochem Parasitol* **73:** 75-90.

Helm R, Cockrell G, Stanley JS, Brenner RJ, Burks W, Bannon GA (1996) Isolation and characterization of a clone encoding a major allergen (Bla g Bd90K) involved in IgE-mediated cockroach hypersensitivity. *J Allergy Clin Immunol* **98:** 172-180.

Hotokezaka Y, Tobben U, Hotokezaka H, Van Leyen K, Beatrix B, Smith DH, Nakamura T, Wiedmann M (2002) Interaction of the eukaryotic elongation factor 1A with newly synthesized polypeptides. *J Biol Chem* **277:** 18545-18551.

Iraneta SG, Duschak VG, Rodriguez SM, Seoane MA, Albonico JF, Alonso A (1999) Proteinase and gelatinolytic activities of house dust mite and cockroach extracts. *J Investig Allergol Clin Immunol* **9:** 235-240.

Kim I, Cha SY, Yoon MH, Hwang JS, Lee SM, Sohn HD, Jin BR (2005) The complete nucleotide sequence and gene organization of the mitochondrial genome of the oriental mole cricket, *Gryllotalpa orientalis* (Orthoptera: Gryllotalpidae). *Gene* **353:** 155-68.

Landais I, Ogliastro M, Mita K, Nohata J, Lopez-Ferber M, Dounor-Cerutti M, Shimada T, Fournier P, Devauchelle G (2003) Annotation pattern of ESTs from *Spodoptera frugiperda* Sf9 cells and analysis of ribosomal protein genes reveal insect-specific features and unexpectedly low codon usage bias. *Bioinformatics* **19:** 2343-2350.

Manger ID, Hehl A, Parmley S, Sibley LD, Marra M, Hillier L, Waterston R, Boothroyd JC (1998) Expressed sequence tags analysis of the bradyzoite stage of *Toxoplasma gondii*: Identification of developmentally regulated genes. *Infect Immun* **66:** 1632-1637.

Martinez-Gonzalez J, Hegardt FG (1994) Cytochrome c oxidase subunit I from the cockroach *Blattella germanica*: cloning, developmental pattern and tissue expression. *Insect Biochem Mol Biol* **24:** 619-626.

Missirlis F, Phillips JP, Jackle H (2001) Cooperative action of antioxidant defense systems in *Drosophila*. *Curr Biol* **11:** 1272-1277.

Ock MS, Kim BJ, Kim SM, Byun KH (2005) Cloning and expression of trypsin-encoding cDNA from *Blattella germanica* and its possibility as an allergen. *Korean J Parasitol* **43:** 101-110.

Ock MS, Kim KR (1989) Karyotype analysis of *Blattella germanica* L. and *Blattella nipponica* Asahina in Korea. *J Kosin Med Col* **5:** 21-25.

Pridgeon JW, Liu N (2003) Overexpression of the cytochrome c oxidase subunit I gene associated with a pyrethroid resistant strain of German cockroaches, *Blattella germanica* (L.). *Insect Biochem Mol Biol* **33:** 1043-1048.

Richman PG, Picone FJ, Strunk RS, So J, Colten HR (1984) The important sources of German cockroach allergens as determined by RAST analyses. *J Allergy Clin Immunol* **73:** 590-595.

Ross MH, Cochran DG (1975) The German cockroach, *Blattella germanica*. In: King RC editor. Handbook of genetics Vol. 3 New York: Plenum. p 35-62.

Scharf ME, Neal JJ, Marcus CB, Bennett GW (1998) Cytochrome P450 purification and immunological detection in an insecticide resistant strain of German cockroach (*Blattella germanica* L.). *Insect Biochem Mol Biol* **28:** 1-9.

Ussery DW, Hallin PF (2004) Genome update: length distributions of sequenced prokaryotic genomes. *Microbiol Comment* **150:** 513-516.

Wen Z, Horak CE, Scott J (2001) CYP9E2, CYP4C21 and related pseudogenes from German cockroaches, *Blattella germanica*: implications for molecular evolution, expression studies and nomenclature of P450s. *Gene* **272:** 257-266.

Wongtim S, Lehrer SB, Salvaggio JE, Horner WE (1993) Protease activity in cockroach and basidiomycete allergen extracts. *Allergy Proc* **14:** 263-268.

Wool IG (1996) Extraribosomal functions of ribosomal proteins, *Trends Biochem Sci* **21:** 164-165.

Wu CH, Lee MF, Liao SC, Luo SF (1996) Sequencing analysis of cDNA clones encoding the American cockroach Cr-PI allergens. Homology with insect hemolymph proteims. *J Biol Chem* **271:** 17937-17943.