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Short Communication

Insights into the *Melipona scutellaris* (Hymenoptera, Apidae, Meliponini) fat body transcriptome

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Abstract

The insect fat body is a multifunctional organ analogous to the vertebrate liver. The fat body is involved in the metabolism of juvenile hormone, regulation of environmental stress, production of immunity regulator-like proteins in cells and protein storage. However, very little is known about the molecular mechanisms involved in fat body physiology in stingless bees. In this study, we analyzed the transcriptome of the fat body from the stingless bee *Melipona scutellaris*. *In silico* analysis of a set of cDNA library sequences yielded 1728 expressed sequence tags (ESTs) and 997 high-quality sequences that were assembled into 29 contigs and 117 singlets. The BLAST X tool showed that 86% of the ESTs shared similarity with *Apis mellifera* (honeybee) genes. The *M. scutellaris* fat body ESTs encoded proteins with roles in numerous physiological processes, including anti-oxidation, phosphorylation, metabolism, detoxification, transmembrane transport, intracellular transport, cell proliferation, protein hydrolysis and protein synthesis. This is the first report to describe a transcriptomic analysis of specific organs of *M. scutellaris*. Our findings provide new insights into the physiological role of the fat body in stingless bees.

Keywords: fat body, gene expression, *Melipona*, stingless bees, transcriptome.

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The insect fat body is a diffuse organ that fills the body cavity and consists of mesodermal cells known as trophocytes or fat body cells (Arrese and Soulages, 2010; Roma *et al.*, 2010). In addition to trophocytes, bees contain oenocytes, another cell type of ectodermal origin found scattered throughout the fat body (Paes de Oliveira and Cruz-Landim, 2003; Martins *et al.*, 2011a,b; Price *et al.*, 2011).

The insect fat body is a multifunctional organ with a role in a variety of metabolic processes, including: a) storage of proteins, lipids and carbohydrates, which are precursors for metabolism in other organs, b) regulation of chemical compounds released in the haemolymph, c) synthesis of vitellogenin and d) synthesis of antimicrobial peptides that act in the innate immune response (Cruz-Landim, 1985; Tzou *et al.*, 2002; Andrade *et al.*, 2010, 2011; Ottaviani *et al.*, 2011).

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Gene expression, assessed via transcriptomic analysis, can provide insights into animal biology and physiology since changes in gene expression may reflect genetic responses to environmental stimuli, as well as immune responses. Expressed sequence tags (ESTs) provide a fast, accurate tool for gene identification and are widely used in functional genomic studies (Adams *et al.*, 1991; Zweiger and Scott, 1997; Martins *et al.*, 2011b).

Melipona scutellaris is a stingless bee found in northeastern Brazil. Populations of this bee have decreased substantially as a consequence of human activity (Kerr *et al.*, 1996) and studies of this species are important for its maintenance.

Workers of *M. scutellaris* were obtained from the stingless bee colony at the Institute of Genetics and Biochemistry, Federal University of Uberlandia, State of Minas Gerais, Brazil. Foraging workers were identified by the presence of pollen in the pollen sacs and were collected when returning to the hive. The abdomens of 15 foragers were opened with forceps while submerged in insect saline solution (0.1 M NaCl, 0.1 M KCl, 0.1 M $CaCl_2$) and the fat

body layers under the tergites and sternites were separated, transferred to liquid nitrogen and stored at -80 °C.

mRNA was purified using a Micro-FastTrack 2.0 mRNA isolation kit (Invitrogen) and used to synthesize the first strand of cDNA. A cDNA library of the *M. scutellaris* fat body was constructed using a SuperScript Plasmid System kit with Gateway Technology for cDNA Synthesis and Cloning (Invitrogen). The clones were sequenced using DYEnamic ET Dye Terminator Cycle Sequencing for MegaBace DNA Analysis Systems kits (GE Healthcare) according to the manufacturers recommendations and analyzed with a MegaBace 1000 sequencer (Amersham Biosciences).

The computer program EGassembler from the Human Genome Center was used to trim the vector and mitochondrial sequences. Phred (Ewing and Green, 1998; Ewing *et al.*, 1998) and Base Caller Cimarron v.3.12 softwares were used to identify high-quality sequences (Phred > 20 and ≥150 base pairs) that were assembled using CAP3. The high-quality sequences were compared using the algorithms BLAST X and BLAST N v.2.2.25 with an initial E-value threshold of 10^{-6} (Altschul *et al.*, 1990, 1997). Gene Ontology and FatiGO tools were used to assign possible biological functions to the fat body transcripts based on comparison with *Drosophila* proteins, as described by Al-Shahrour *et al.* (2004).

A total of 2.26×10^5 clones were obtained from the fat body cDNA library. The length of randomly cloned cDNAs was determined by agarose gel electrophoresis; the fragment size varied from 150 to 2000 base pairs (data not shown).

The fat body cDNA sequences yielded 1728 ESTs and 997 high quality reads longer than 150 bp, totaling 197,904 nucleotides. Of the 997 high quality reads, 392 (39.3%) ESTs were from mitochondrial genes and the remaining 605 (60.7%) were edited and assembled into 29 contigs and 117 singlets. Seventy-one percent of the ESTs showed matches with the transcriptome shotgun assembly from *Melipona quadrifasciata* obtained by Woodard *et al.* (2011). The ESTs identified here were deposited in dbEST under accession numbers HO000185-HO000320 and HO000363-HO000419.

Nearly 40% of the *M. scutellaris* fat body ESTs were of mitochondrial origin and are likely also found in the fat body of *Aedes aegypti* (Feitosa *et al.*, 2006). The number of mitochondria per cell increases 700 fold in newly emerged adult insects (Kurella *et al.*, 2001), suggesting that the mitochondria of fat body cells are an important source of energy for adult bees. In bees, the fat body plays a role in protein synthesis and storage (Ivanova and Staikova, 2007) and, in agreement with this, we found transcripts involved in protein synthesis.

The *M. scutellaris* fat body ESTs showed 86% similarity with *Apis mellifera* genes and the remaining 14% with *Nasonia vitripennis*, *Drosophila melanogaster* and others (Figure 1). Of the two hymenopteran species, *A. mellifera* is phylogenetically closer to *M. scutellaris* than *N. virtripennis* (The Honeybee Genome Sequencing Consortium, 2006; Nasonia Genome Working Group, 2010).

The comparison of contigs and singlets with their respective orthologs in *D. melanogaster* showed that the transcripts identified in *M. scutellaris* fat bodies were associated with known biological processes, cellular components and molecular functions (Figure 2). The main functions of these proteins were anti-oxidation, phosphorylation, metabolism, detoxification, transmembrane transport, intracellular transport, cell proliferation, protein hydrolysis and protein synthesis. The contigs were assembled from overexpressed genes and most were found to encode proteins with roles in cell metabolism (Table 1). These data agree with the physiological role of the insect fat body (Price *et al.*, 2011).

The BLASTX and BLASTN analyses identified sequences homologous to proteins associated with the immune response (Toll proteins, kinases and cytochrome P450), resistance to insecticides, cell cycle control, juvenile hormone metabolism and resistance to environmental stress.

The insect fat body is an organ of the immune system and we identified ESTs related to the immune response in the fat bodies of *M. scutellaris*, *e.g.*, *Toll* and other genes, as well as genes for cytochrome P450 and kinases. Martins *et al.* (2011a) found *P450* in *A. aegypti* oenocytes, a cell type

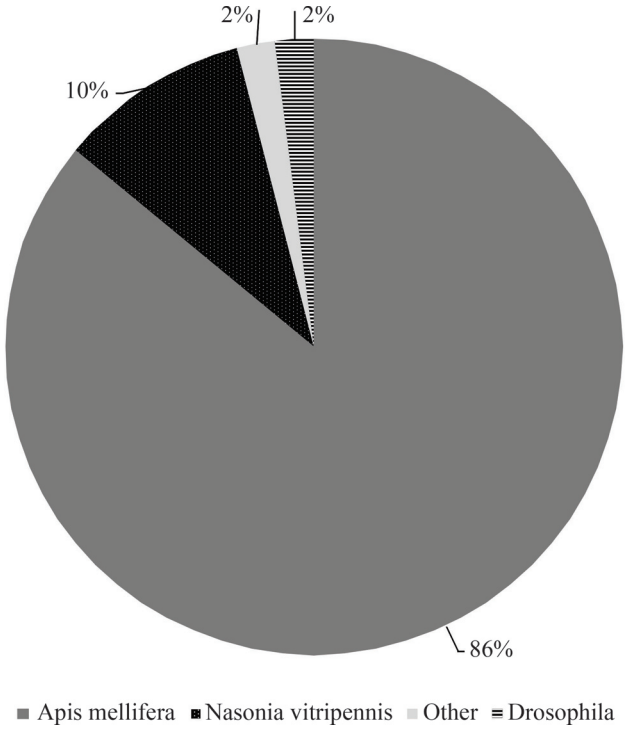

Figure 1 - Transcript distribution of the best matches of the *Melipona scutellaris* fat body cDNA library. The best matches were listed based on the insect species without considering E-values.

scattered amongst trophocytes in bees. Insects are resistant to infection by microorganisms, although an acquired immune system is lacking. The immune response of insects consists of an innate immune system in which microorganisms and molecules are recognized by specific receptors, thereby activating the cellular immune response associated with phagocytosis, encapsulation and humoral responses (Ribeiro and Brehélin, 2006; Andrade *et al.*, 2010). Hemo-

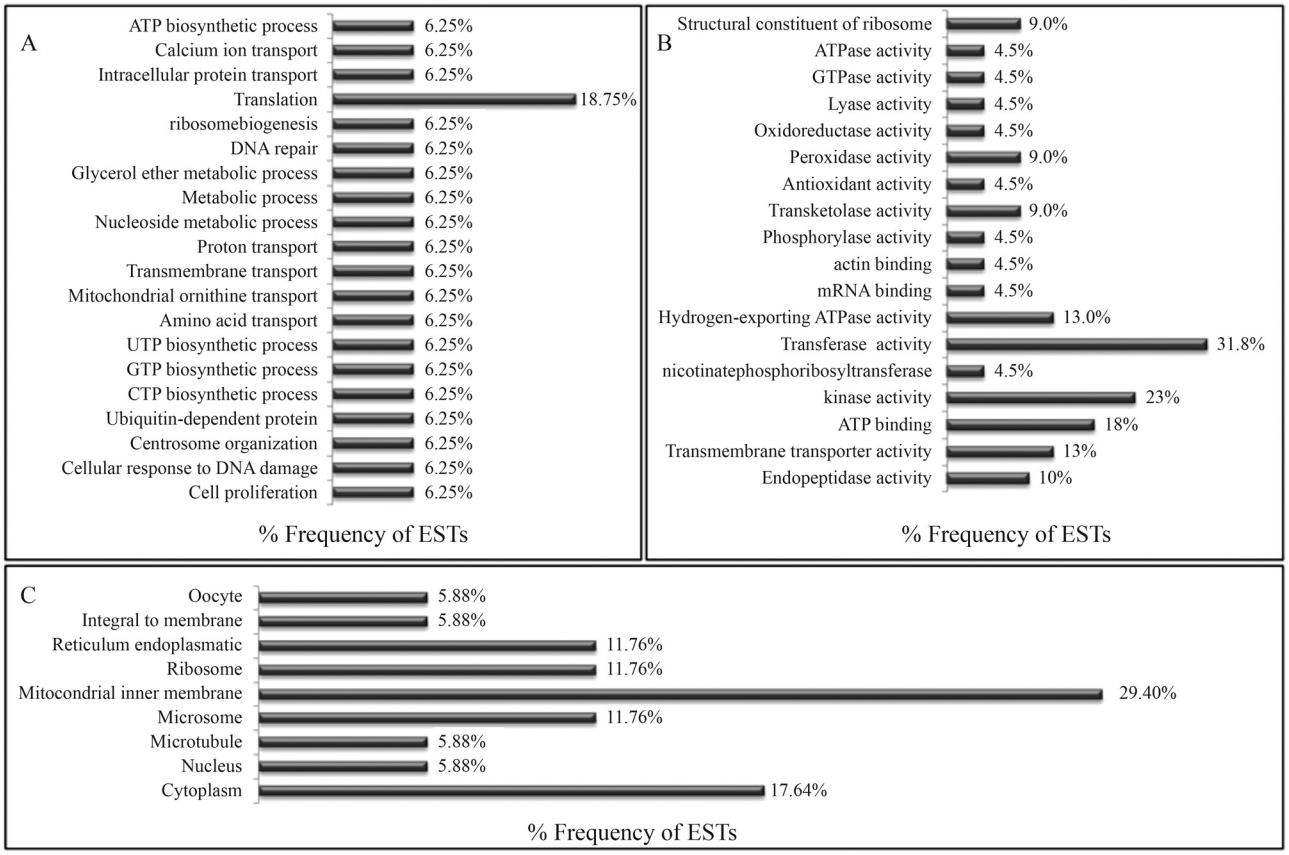

Figure 2 - Transcript distribution of *Melipona scutellaris* fat body cDNA library. Protein functions were assigned based on homology with *Drosophila* genes. (A) Biological process. (B) Molecular function. (C) Cell component.

Table 1 - Best matched *Melipona scutellaris* contigs from BlastN analysis. The *Melipona* contig ID is the access number in GenBank. Only sequences that matched the insect sequence are shown.

Melipona contig ID	Best match	E-value	Identity (%)
HO000238.1	PREDICTED: similar to CG7530-PA, isoform A [*Apis mellifera*]	4.00E-18	90
HO000202.1	PREDICTED: similar to CG4692-PB, isoform B [*Apis mellifera*]	1.00E-46	68
HO000240.1	PREDICTED: similar to translationally controlled tumor protein [*Nasonia vitripennis*]	9.00E-15	76
HO000411.1	PREDICTED: similar to F27C1.2a [*Apis mellifera*]	1.00E-10	54
HO000230.1	PREDICTED: similar to CG10672-PA [*Apis mellifera*]	2.00E-19	63
HO000262.1	PREDICTED: similar to CG3271-PB, isoform B [*Apis mellifera*]	3.00E-11	79
HO000263.1	PREDICTED: *Apis mellifera* putative fatty acyl-CoA reductase CG5065-like	3.00E-33	72
HO000317.1	Heat shock protein 90 [*Apis mellifera*]	3.00E-16	71
HO000312.1	PREDICTED: similar to R04B5.5 [*Apis mellifera*]	2.00E-71	100
HO000199.1	PREDICTED: similar to sentrin/sumo-specific protease senp7 [*Nasonia vitripennis*]	5.00E-10	58
HO000273.1	PREDICTED: similar to serine/threonine-protein kinase rio1 [*Nasonia vitripennis*]	4.00E-18	64
HO000373.1	Farnesoic acid o-methyltransferase-like isoform 1 protein [*Melipona scutellaris*]	4.00E-34	100
HO000393.1	PREDICTED: similar to microsomal glutathione S-transferase-like CG1742-PA, isoform A isoform 1 [*Apis mellifera*]	4.00E-54	70
HO000389.1	GJ13450 [*Drosophila virilis*]	6.00E-06	53

cytes are cells that play a role in the cellular immune response of insects and have been described in *M. scutellaris* larvae (Amaral *et al.*, 2010).

Insects can recognize specific microbial markers known as pathogen-associated molecular patterns (PAMPs). PAMPs are recognized by pattern recognition receptors (PRR) that mediate cellular immune responses such as phagocytosis and the serine-proteinase cascade that activates melanization and/or the release of antimicrobial substances (Kavanagh and Reeves, 2004). A PRR found in insects is Toll, a transmembrane receptor with a leucine-rich extracellular domain and an intracellular region similar to the interleukin-1 receptor (Leclerc and Reichhart, 2004). Toll receptors recognize lipids, carbohydrates, peptides and nucleic acids from different invaders (Akira *et al.*, 2006).

Amongst the *M. scutellaris* fat body ESTs, 23% were for kinases, mainly mitogen-activated protein kinases (MAP-kinases). Protein kinases (PKs) play a central role in signal transduction, including the transmission of environmental stimuli, the coordination of intracellular processes and in invertebrate defense against pathogens (Kim *et al.*, 2002; Wojda *et al.*, 2004; Chen Chih *et al.*, 2007). In addition, MAP-kinases, such as p42/44 ERK, p38 MAPK, JNK, PKA, PKB, PKC and Akt are involved in the control of cell apoptosis (Cross *et al.*, 2000).

Among the transcripts identified in this study we found an EST that encoded cytochrome P450 (CYP6g2), a family of enzymes with multiple functions (Mansuy, 1998). Although numerous P450 isoforms have been identified in insects, with an average of 80-120 isoforms per individual (Tijet *et al.*, 2001), *A. mellifera* has only 46 genes that encode P450 proteins (Claudianos *et al.*, 2006). In *Drosophila*, the genes Cyp12d1, Cyp6g1 and Cyp6g2 are associated with resistance to DDT, neonicotinoids and growth regulators (Daborn *et al.*, 2007). In the *M. scutellaris* fat body, the EST that encoded for P450 was an ortholog of *D. melanogaster* CYP6g2, which suggested a function in resistance to insecticides in this stingless bee.

Heat shock proteins (HSPs) have various functions in combating environmental stress and a variety of agents, such as temperature, dehydration, chemicals, heavy metals and other xenobiotics can regulate the expression of HSP (Sun and MacRae, 2005; Rinehart *et al.*, 2006; Benoit *et al.*, 2010). Several ESTs for HSPs were detected in the *M. scutellaris* fat body transcriptome. Two of these ESTs showed high similarity (92%; E-value = 7 x 10^{-77}) with cytosolic HSP90 isoforms that have been associated with caste differentiation in *A. mellifera* (Xu *et al.*, 2010). Some genes associated with caste differentiation in honeybees have been identified (Pinto *et al.*, 2002; Cristino *et al.*, 2006; Barchuk *et al.*, 2007; Mackert *et al.*, 2010), and caste differentiation in stingless bees has also been widely studied (Kerr, 1947; Kerr and Nielsen, 1966; Bonetti, 1984, 1995; Santana *et al.*, 2006; Vieira *et al.*, 2008).

An EST found in the fat body of *M. scutellaris* was similar to a putative farnesoic acid O-methyl-transferase (FAMeT). This putative enzyme catalyzes the synthesis of methylfarnesoate from farnesoic acid in the biosynthetic pathway of juvenile hormone in the *corpora allata*. Vieira *et al.* (2008) found that FAMeT of *M. scutellaris* (MsFAMeT) has different expression levels in castes, suggesting that this enzyme may be associated with the metabolism of juvenile hormone in this stingless bee. These findings suggest either that this enzyme is ambiguous in *Melipona* and can participate in other biochemical pathways or that it is not specific for juvenile hormone synthesis. Although no ESTs encoding for enzymes that degrade juvenile hormone were detected in the *M. scutellaris* fat body, RT-PCR and qPCR detected mRNAs for juvenile hormone esterase and juvenile hormone epoxide hydrolase (data not shown), which confirms the expression of these genes in this fat body.

In conclusion, we have identified 1728 ESTs from the fat body of *M. scutellaris*. *In silico* analysis of these ESTs has provided new insights into the physiological roles of this tissue in phenomena such as innate immunity, cellular proliferation, resistance to insecticides and environmental stress, and caste differentiation in stingless bees.

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