

Elevated expression of ageing and immunity genes in queens of the black garden ant.

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Electronic Supplementary Material

Supplementary information on the annotation of comp82602_c1.

Annotation of SOD3

With individuals of both ages combined, the most significantly caste-biased gene in brains was comp82602_c1. A blast of this gene's ORF against the Swissprot proteomes identified it as a homolog of CuZn Superoxide Dismutase (CuZn-SOD) in *D. melanogaster*. However, the top reciprocal blast of CuZn-SOD against *L. niger* did not return comp82602_c1 as its top hit, indicating that comp82602_c1 is not the closest homolog of *D. melanogaster* CuZn-SOD in the *L. niger* transcriptome.

Two homologs forms of CuZn SOD have been described in *L. niger*, a cytosolic (SOD1) and an extra-cellular (SOD3) form (Parker et al. 2004), hinting that comp82602_c1 could represent SOD3. We blasted the sequences for *L. niger* SOD1 (AY309973.1) and SOD3 (AY672457.1) against the *L. niger* transcriptome. This identified SOD1 as the isotig comp74197_c0_seq1 (100% identity over an alignment of 462 bases) and SOD3 as the isotig comp82602_c1_seq1 (99.7% identity over an alignment of 1011 bases).

Alignment of the predicted protein sequence for the Open Reading Frame (ORF) of comp82602_c1_seq1 (SOD3) against the two *Drosophila* isoforms of SOD3 (AIU57094.1 and AIU57095.1, Blackney et al. 2014) reveal that the gene identified here is the short, non-membrane associated isoform of SOD3 (AIU57094.1), since it does not share the hydrophobic tail of the membrane associated form (AIU57095.1):

LnigerSOD3_ORF	-MSRMVALLLLAAVTTVTAE--E---MVAVVSLTPHNVKEKNVTGNLKIIVQSVPNGPVII
AIU57094.1	MMQYLVVSLALCATICSAAQTRNMPIQAIAYLIGPVQSDNTQVKGNVTFTQNDCGQNVHV
AIU57095.1	MMQYLVVSLALCATICSAAQTRNMPIQAIAYLIGPVQSDNTQVKGNVTFTQNDCGQNVHV *. :*. * *.*. :*: : . . : * : .:.:*.***:..* . *
LnigerSOD3_ORF	TGTIHGLTEGLHGFHVHEKGDLSDGCTSAGAHFNPDNVTHTGAPEDTVRHVGDLGNIQANS
AIU57094.1	RVQLEGLKEGKHGFHIHEKGDLTNGCISMGAHYNPDKVDHGPGDHEVRHVGD LGNLEANS
AIU57095.1	RVQLEGLKEGKHGFHIHEKGDLTNGCISMGAHYNPDKVDHGPGDHEVRHVGD LGNLEANS :.*.*.*** *****:*****:* * ***:**:* **,*;. *****:***
LnigerSOD3_ORF	EGEATVNITDSMISLTGPNNILGRSIVVHSGEDDLGKGNHLSSTTGNSGSRWACGVIGV
AIU57094.1	TGIIDVTYTDQVITLTGKLGIIGRGVVVHELED DLGLGNHTDSKKTGNAGGRIACGVIGI
AIU57095.1	TGIIDVTYTDQVITLTGKLGIIGRGVVVHELED DLGLGNHTDSKKTGNAGGRIACGVIGI * *. **.:**:* .*:*.:****. ***** **: *.****:* * ****:
LnigerSOD3_ORF	QKI-----
AIU57094.1	K-----
AIU57095.1	NSDVDEWPCRDGGAGALRYSFSILT VIVALIMARSLD :

This alignment was produced using the Clustal omega website:
<http://www.ebi.ac.uk/Tools/msa/clustalo> with default parameters.

Parker, J. D.; Parker, K. M. & Keller, L. (2004). *Molecular phylogenetic evidence for an extracellular Cu Zn superoxide dismutase gene in insects*, Insect molecular biology 13 : 587-594.

Blackney, M. J.; Cox, R.; Shepherd, D.; Parker, J. D. (2014). *Cloning and expression analysis of Drosophila extracellular Cu Zn superoxide dismutase*, Bioscience Reports 34 : 851-863.