

Gene expression profiling of undertaking behavior in the eastern subterranean termite, Reticulitermes flavipes

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Introduction

Undertaking behavior, the disposal of dead individuals to prevent potential pathogenic attack, is an essential adaptation to social living. It has convergently evolved in the honey bee, ants and termites ^[1]. Unlike ants and bees, which typically remove corpses out of nest, in the eastern subterranean termite, *Reticulitermes flavipes*, workers cannibalize freshly dead nestmates, but bury decayed nestmates as well as corpses of competitive species ^[2, 3]. However, the molecular basis of undertaking behavior remains unknown.



This study aims to investigate the molecular signatures of undertaking behavior in *R. flavipes*. The results will advance our understanding of social behavior in termites, and provide candidate molecular targets for the control of this wood-feeding insect pest.

Objectives

- Investigate gene expression profiles in response to different types of corpses;
- Comparatively analyze transcriptional signatures associated with different undertaking behaviors.

Materials and Methods

Insects: *Reticulitermes flavipes* (Kollar)

Treatments: Groups of 20 workers and 1 soldier

Figure 2. Behavioral profiling. Workers responded differently toward the three types of corpses. They cannibalized freshly dead nestmates, buried decayed nestmates, and buried dead competitors.



Figure 3. Differentially expressed genes (DEGs) in response to three **types of corpses.** Relatively, more DEGs (Log₂(fold-change) ≥1, FDR ≤0.001) were identified during burial behavior than cannibalism of nestmates.

A: Number of DEGs

involved in metabolic pathways, particularly carbohydrate metabolisms, were identified in burial behavior. There were also more DEGs involved in sensory, digestive, and immune systems during burial than cannibalism. Data were pooled from 30 min, 1 h and 6 h samples for each treatment.

Discussion

Consuming dead nestmates is beneficial to colony because of nutrient recycling, but burial may be necessary with increased risks such as potential pathogenic attack and colony intrusion. Burial is costly in terms of energy and metabolic resources. During corpse burial, termites secrete saliva containing antimicrobial peptides, which is mixed with soil/sand for disposal of the dead. Our results of DEGs in carbohydrate metabolism, digestive and immune systems reflected the difference in energy output between cannibalism and burial.

(Fig. 1) were exposed to (1) freshly dead nestmates, (2) decayed nestmates, or (3) freshly dead competitors (*R. malettei*). After corpse introduction, the heads of workers were collected at 30 min, 1 h, and 6 h, respectively. In the control group, termites were not exposed to corpse.



RNA-Seq: RNAs extracted from worker heads were sequenced via Illumina by BGI (Beijing) Genomics Institute).

Bioinformatics: Genes differentially expressed compared to control were identified, and annotated through Gene Ontology (GO) and Kyoto **Encyclopedia of Genes and Genomes (KEGG)** pathway analysis using Blast2GO.



11

21

B: Number of GO terms



Cannibalism

Burial of nestmates

Burial of competitors

Figure 4. Comparison of genes, functions, and pathways in differential **undertaking behavior.** Among the three undertaking processes, there was only a small portion of DEGs in common; however, more shared DEGs were identified between the two burial behaviors (A). In addition, a substantial overlap in terms of gene function (**B**) and biological pathway (**C**) was found between burial of nestmates and burial of competitors. Data were pooled from 30 min, 1 h and 6 h samples for each treatment.

Our future work is to test the function of candidate genes using RNAi, and elucidate the genetic underpinnings of undertaking behavior in termites.

Conclusions

- 1. Death cues cause immediate changes (30 min) in gene expression in workers.
- 2. Different sets of DEGs are associated with each type of undertaking response.
- 3. In comparison to cannibalism, burial of nestmates and burial of competitors involve more DEGs associated with metabolic pathways, suggesting higher energetic output in burial behavior. This is consistent with our behavioral observation.

References

- [1] Sun Q & Zhou X. International Journal of Biological Sciences. (2013) 9: 313.
- [2] Neoh KB, Yeap BK, Tsunoda K, et al. PLoS One. (2012) 7: e36375.
- [3] Sun Q, Haynes KF & Zhou X. Scientific reports. (2013): 3: doi:10.1038/srep01650

