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PP-406 [Bee Biology]

# Difference between uncapping and removal behaviors in *Apis cerana* from the perspective of long non-coding RNAs

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## Background/Purpose

Hygienic behavior is a critical immune response in social insects, essential for protecting colonies from disease. In honeybee colonies, this behavior involves two distinct steps: uncapping and removal of unhealthy or deceased brood. While previous research has explored the development of hygienic behavior, the mechanisms driving the division of labor between uncapping and removal remain poorly understood. Long non-coding RNAs (lncRNAs) have been implicated in regulating honeybee physiology, but their role in these specific tasks has not been investigated. This study aims to elucidate the differences between uncapping and removal behaviors in Apis cerana from the perspective of lncRNAs.

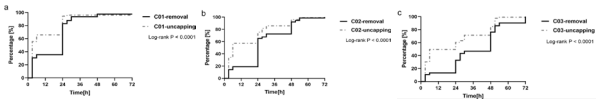
## Methodology

We used strong hygienic *A. cerana* worker bees and assessed uncapping and removal behaviors in three colonies using freeze-killed brood. Antennal RNA sequencing was performed to identify differentially expressed lncRNAs, followed by lncRNA-mRNA association analysis to explore the molecular differences between the two behaviors.

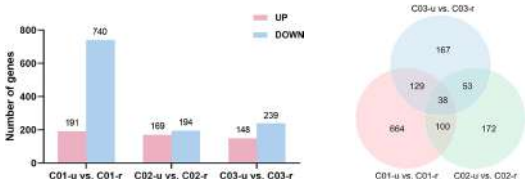
## Results and Conclusion

### 1. Data recording and statistical analyses of freeze-killed broods

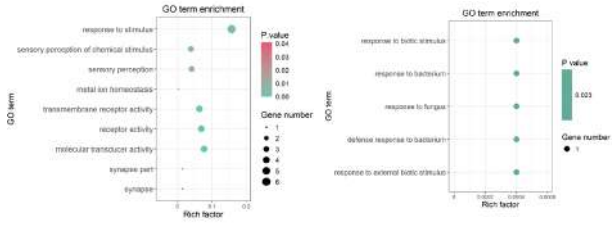
For all of the assessed colonies, the results revealed significant differences in task performances of uncapping and removal worker bees ($P < 0.0001$).



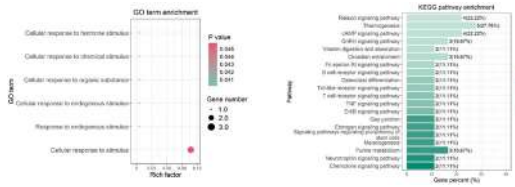
### 2. RNA sequencing of honeybee antennae




### 3. GO analysis of DElncRNAs via antisense and *cis* regulatory mechanisms




### 4. Analysis of the core target genes regulated by common DElncRNAs between "uncapping" and "removal" bees




We identified 1,323 differentially expressed lncRNAs in the antennae. The lncRNA-mRNA association analysis revealed that target genes of these lncRNAs were primarily associated with response to stimulus, receptor activity, and synapse. Notably, the lncRNA XR_001766094.2, enriched in cellular response to stimulus, was exclusively expressed in uncapping worker bees. This suggests that XR_001766094.2 may play a key role in distinguishing uncapping from removal behaviors by modulating responsiveness to external stimuli. Our findings highlight distinct molecular profiles underlying uncapping and removal behaviors, with uncapping bees potentially exhibiting faster stimulatory responses and heightened olfactory sensitivity. These differences may facilitate the rapid execution of hygienic behavior in colonies. This study provides a hint for understanding lncRNA-mediated gene regulation in hygienic behavior.

## Practical Implications for Beekeepers

Understanding the molecular basis of hygienic behavior could aid in breeding programs to enhance colony health and disease resistance. XR_001766094.2 emerges as a promising molecular genetic marker that can potentially assist this targeted breeding endeavor.

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Problem Description: Despite the importance of hygienic behavior in colony health, the molecular mechanisms underlying the division of labor between uncapping and removal tasks are unclear. Understanding these mechanisms could provide insights into how honeybees coordinate complex behaviors to maintain colony hygiene.

METHODOLOGY: We used strong hygienic A. cerana worker bees and assessed uncapping and removal behaviors in three colonies using freeze-killed brood. Antennal RNA sequencing was performed to identify differentially expressed lncRNAs, followed by lncRNA-mRNA association analysis to explore the molecular differences between the two behaviors.

Results and CONCLUSION: We identified 1,323 differentially expressed lncRNAs in the antennae. The lncRNA-mRNA association analysis revealed that target genes of these lncRNAs were primarily associated with response to stimulus, receptor activity, and synapse. Notably, the lncRNA XR_001766094.2, enriched in cellular response to stimulus, was exclusively expressed in uncapping worker bees. This suggests that XR_001766094.2 may play a key role in distinguishing uncapping from removal behaviors by modulating responsiveness to external stimuli. Our findings highlight distinct molecular profiles underlying uncapping and removal behaviors, with uncapping bees potentially exhibiting faster stimulatory responses and heightened olfactory sensitivity. These differences may facilitate the rapid execution of hygienic behavior in colonies. This study provides a hint for understanding lncRNA-mediated gene regulation in hygienic behavior.

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