Bumblebee Foraging Specialization and Expression Levels of a cGMP-related Protein Kinase

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INTRODUCTION

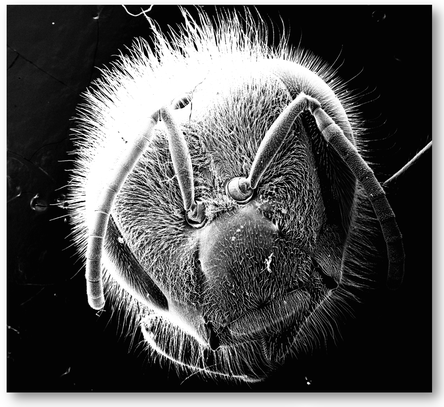
The behavioral specialization of individual workers is a key reason for the ecological success achieved by eusocial insects—those that live in cooperative colonies with queens and workers. Just as humans are trained as doctors, carpenters, or fishermen; eusocial insects, like honeybees (*Apis mellifera)* divide their tasks to specific members of the colony in order to maximize resources and allocate energy efficiently. For example, honeybees have in-nest workers and out-of-nest foragers and amongst the foragers some specialize on gathering pollen, while others gather nectar.

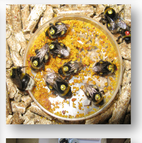
- Foraging behavior in honeybees has also been linked to a gene called *foraging*, but only by the difference in expression in nurses (in-hive workers) and foragers.
- Recent research has shown that despite their smaller and simpler colonies, bumblebees (*Bombus impatiens*) have exhibited the same specialization behavior. Bumblebees have an analogue to *foraging*, a protein kinase, or more simply put, a signaling protein. This gene influences foraging behavior in a general sense, but not in the context relating to specialization.

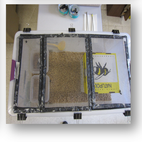
This summer we observed foraging trips of bumblebees to pollen and nectar feeders. We categorized individuals as nectar specialists, pollen specialists, or generalists. We then measured the expression levels of the putative *foraging* gene to test whether it influences foraging specialization or not as seen in honeybees. Similar gene expression patterns would suggest shared regulatory mechanisms across the two groups, while differential expression patterns would suggest that bumblebees and honeybees evolved different mechanisms for foraging specialization.

HYPOTHESIS

Expression levels of the cGMP-related protein kinase in *B. impatiens* influences individual bee foraging specialization.

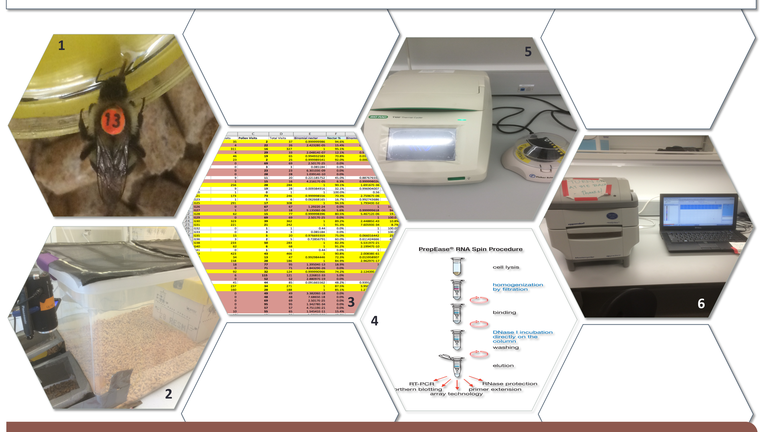




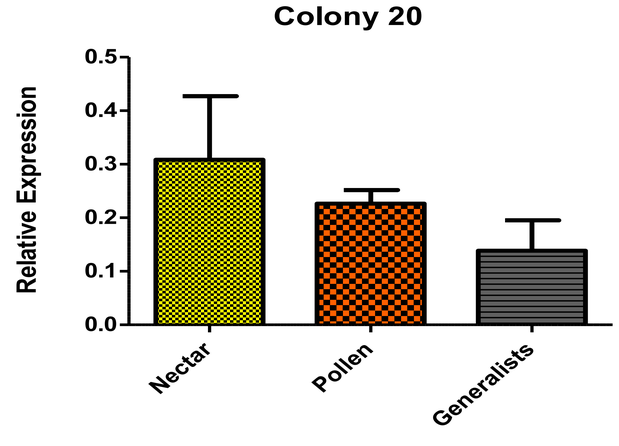


MATERIALS & METHODS

1. **Marking-** Marked all bees using colored and numbered tags.
2. **Observations-** Videotaped all foraging bees at pollen and nectar feeders.
3. **Analysis-** Determined specialists using the binomial test.
4. **Extraction-** Extracted RNA from individual bee heads using the PrepEase® RNA spin kit.
5. **Conversion**- Converted RNA into cDNA using High Capacity cDNA Reverse Transcription Kit and Bio-Rad T-100® Thermal Cycler.
6. **qPCR-** Using primers for the cGMP-related protein kinase, which is 99% similar to *foraging* in *B. terrestris*, did qPCR, or quantitative Real-Time PCR with EXPRESS SYBR® Green-ER Supermix and Eppendorf® realplex^2 Mastercyler to amplify the target sequence and evaluate its expression level in foragers.



RESULTS



Graph 1-Relative Expression Levels of nectar specialists, pollen specialists and generalists in colony 20

RESULTS

Graph 2-Relative Expression Levels of nectar specialists, pollen specialists and generalists in colony 21

STATISTICAL ANALYSIS

Colony 20

ANOVA $F_{2,8}$=1.1; p=0.38

Colony 21

ANOVA $F_{2,10}$=4.9; p=0.03

CONCLUSIONS

1. While we did not observe any upregulation in the *foraging* analogue compared to the constitutively expressed housekeeping gene, we did see differential expression between generalists and specialists (Graphs 1 and 2). Only the nectar to generalist comparison in colony 21 was significant, but the sample size (24) was most likely to blame for this.
2. Overall, there was slight downregulation of the gene of interest, but the downregulation of generalists was greater than that of specialists. Possible error again would be sample size, and the way in which bees' heads were collected.
3. This data tells us that the *foraging* analogue has some influence on bumblebee foraging behavior in individuals between nectar specialists and generalists in one colony. We cannot draw any other significant conclusions, but with a larger sample size we may be able to gather more precise and conclusive data.

ACKNOWLEDGEMENTS

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