

## Honey bees may inherit altruistic behavior from their mothers

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Honey bee workers display altruism by spreading the pheromones of the queen bee and suppressing their own reproduction. Credit: Sean Bresnahan



True altruism is rare behavior in animals, but a new study by Penn State researchers has found that honey bees display this trait. Additionally, they found that an evolutionary battle of genetics may determine the parent from whom they inherit it.

For the study, published in the journal <u>Molecular Ecology</u>, the researchers examined the genetics behind "retinue" behavior in worker honey bees, who are always female. After the <u>worker bees</u> are exposed to the queen bee's pheromone, they deactivate their own ovaries, help spread the pheromone to the other worker bees, and tend to the queen and the eggs she produces.

This behavior is considered altruistic because it ultimately benefits the ability of the queen to produce offspring, while the worker bee remains sterile. For honey bees, the queen is typically the mother of all—or nearly all—the bees in the hive.

The researchers found that the <u>genes</u> that make worker bees more receptive to this pheromone—and therefore more likely to display the retinue behavior—can be passed down from either the mother or father bees. However, the genes only result in <u>altruistic behavior</u> when they are passed down from the mother.

Sean Bresnahan, corresponding author, doctoral candidate in the Intercollege Graduate Degree Program in Molecular, Cellular, and Integrative Biosciences and a National Science Foundation Graduate Research Fellow, said that in addition to giving insight into bee behavior, the findings also show that which parent a bee inherits certain genes from can affect how those genes are expressed, something that is notoriously difficult to study in insects.

"People often think about different phenotypes being the result of differences in gene sequences or the environment," he said. "But what



this study shows is it's not just differences in the gene itself—it's which parent the gene is inherited from. By the very nature of the insect getting the gene from its mom, regardless of what the gene sequence is, it's possibly going to behave differently than the copy of the gene from the dad."

Christina Grozinger, co-author and Publius Vergilius Maro Professor of Entomology at Penn State, said the study also supports the Kinship Theory of Intragenomic Conflict—a theory that suggests the mothers' and fathers' genes are in conflict over what behaviors to support and not support.

She said that while previous work has shown that genes from males can support selfish behavior in mammals, plants and honey bees, the current study is the first to show that genes from females can pass altruistic behavior onto their offspring.

"Honey bees are one of the few animal species that display altruistic behavior, where some individuals give up their own reproduction to help others," Grozinger said. "This study reveals a very subtle and unexpected form of genetic control of those behaviors. With our system, we see that genes from the mother—the queen—are supporting altruistic behavior in her offspring, which leads to more copies of her genes in the population. Instead of producing their own eggs, the worker bees support the queen's reproduction. This complements our previous studies, which showed that the fathers' genes support selfish behavior in worker bees, where the bees will stop helping their queen mother and focus on their own reproduction."

The queen mates with multiple males, so worker bees have the same mother but different fathers. Breshnahan explained that this means they share more of their mother's genes with each other.



"This is why the Kinship Theory of Intragenomic Conflict predicts that genes inherited from the mother will support altruistic behavior in honey bees," Breshnahan said. "A worker bee benefits more from helping, rather than competing with her mother and sisters—who carry more copies of the worker's genes than she could ever reproduce on her own. In contrast, in species where the female mates only once, it is instead the father's genes that are predicted to support altruistic behavior."

For the study, the researchers cross-bred six different lineages of honey bees—something that is relatively easy in mammals or plants, according to Bresnahan, but much harder to do with insects. He said the study wouldn't have been possible without the honey bee breeding expertise of co-author Juliana Rangel at Texas A&M University, as well as Kate Anton who runs the Education about Production and Insemination of (honey bee) Queens program with Robyn Underwood at Penn State Extension.

After the bee populations were crossed and the offspring were old enough, the researchers assessed the worker bees' responsiveness to the pheromone that triggers the retinue behavior, as well as whether the bees deactivated their ovaries in response to the pheromone.

"Finally, we used RNA sequencing to look at genome-wide gene expression in the workers, but importantly, we also sequenced the genomes of the parents of those crosses," Bresnahan said. "So, we could develop personalized genomes for the parents, and then map back the workers' gene expression to each parent and find out which parent's copy of that gene is being expressed."

To try to visualize this conflict happening within the genome, Bresnahan said they used different techniques including machine learning to examine gene regulatory networks, or groups of genes regulated by similar transcription factors to produce similar expression patterns. The



researchers looked at relationships between genes and transcription factors—the proteins that can turn genes on or off—were expressed from the mom's copy and those that were expressed from the dad's copy to identify where one might try to counteract the effects of the other.

Ultimately, they were able to identify gene <u>regulatory networks</u> with intragenomic conflict, finding that more genes were expressed with a parental bias. This maternal- or paternal-originated expression bias is the signature of intragenomic conflict, and the researchers said it appeared more often than it would have if they had constructed the networks with randomly selected genes. Additionally, these networks consisted of <u>genes</u> that previous research showed were related to the retinue behavior.

"Observing intragenomic conflict is very difficult, and so there are very few studies examining the role it plays in creating variation in behavior and other traits," Grozinger said, pointing to the groups prior research that revealed <u>ovary activation</u> and <u>aggression</u> in worker bees, both of which signify selfish behavior.

"The fact that this is the third <u>behavior</u> where we have found evidence that intragenomic conflict contributes to variation in honey bees suggests that intragenomic conflict might shape many types of traits in <u>bees</u> and other species. Hopefully, our research will provide a framework and inspiration for other scientists to examine intragenomic conflict in their plant and animal species," Grozinger added.

David Galbraith, Penn State alumni and senior scientist at Janssen Pharmaceutical; Rong Ma, Penn State alumni and senior data scientist at Visa; Kate Anton, research technologist at Penn State; and Juliana Rangel, Texas A&M University, also collaborated on this work.

**More information:** Sean T. Bresnahan et al, Beyond conflict: Kinship theory of intragenomic conflict predicts individual variation in altruistic



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