

REVIEW

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# Special recipe from pollens for the caste determination of honeybees



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## Abstract

Honeybee caste differentiation is regulated by multiple factors. Here we comment an article published on August 31, 2017, *Plos Genetics*, which show that miRNAs from pollen may affect honeybee caste formation. Through directly silencing *amTOR* activity, pollen miR162a can affect ovary development and determine the fate of larvae to differentiate to worker bees. Reciprocally, miRNAs could also regulate flower development to attract more insects in certain plant species. In addition, their finding provides a novel angle to investigate the co-evolution between plants and insects and gives a clue to explain a recent trend of mysterious death of wild honeybees. Therefore, further exploring miRNAs' function in plant-insect co-evolution is potentially valuable for enhancing agricultural yields.

**Keywords:** Honey bee, Caste, microRNA

## Main text

There is an adage “You are what you eat”, and it means the food may become part of you. In a recent finding published on August 31, 2017, *Plos Genetics*, scientists from School of Life Sciences, Nanjing University proved this adage is literally true in female honeybee. Different food may determine honeybee larvae to develop into distinct fates as either a queen or worker bees. It is known that larvae are not born as a queen or a worker. Instead, larvae fed on royal jelly, a kind of granular secretion by nurse bees, develop into queens. By contrast, those honeybees fed on bee bread composed of pollen and honey will develop into workers [1–4].

In this study, a new mechanism is found that miRNAs (miRNAs) in pollens can affect honeybee genes which regulate ovary development and keep the bees sterile. Further evidence showed that pollen contains various miRNAs, including miR162a, which can directly target and silence *amTOR* activity in honeybee, and regulate ovary development. This helps to establish the caste (Fig. 1). This finding agrees with previous studies that reducing *amTOR* activity in queen destined larvae will turn them into bees with worker characteristics [5–7].

To explore whether miRNAs regulate growth of other insects, the authors tested *Drosophila*. When fed with microRNA-rich diet, *Drosophila* larvae failed to develop properly. The larvae body size, ovary size and fecundity are all compromised. The authors found that miR162a could target a key developmental gene named *dmTOR* in *Drosophila* [8]. It would be interesting if the authors test more insect species to further investigate that pollen miRNAs' role in other insects' development. Depend on the conservation of target genes and the amount of miRNA ingested, it is highly possible that pollen miRNAs would have pleiotropic effects on other insects' development. For example, it would be an interesting question to investigate the role of plant miRNAs in honeybee's close relative, bumblebee, which don't have an obvious caste formation.

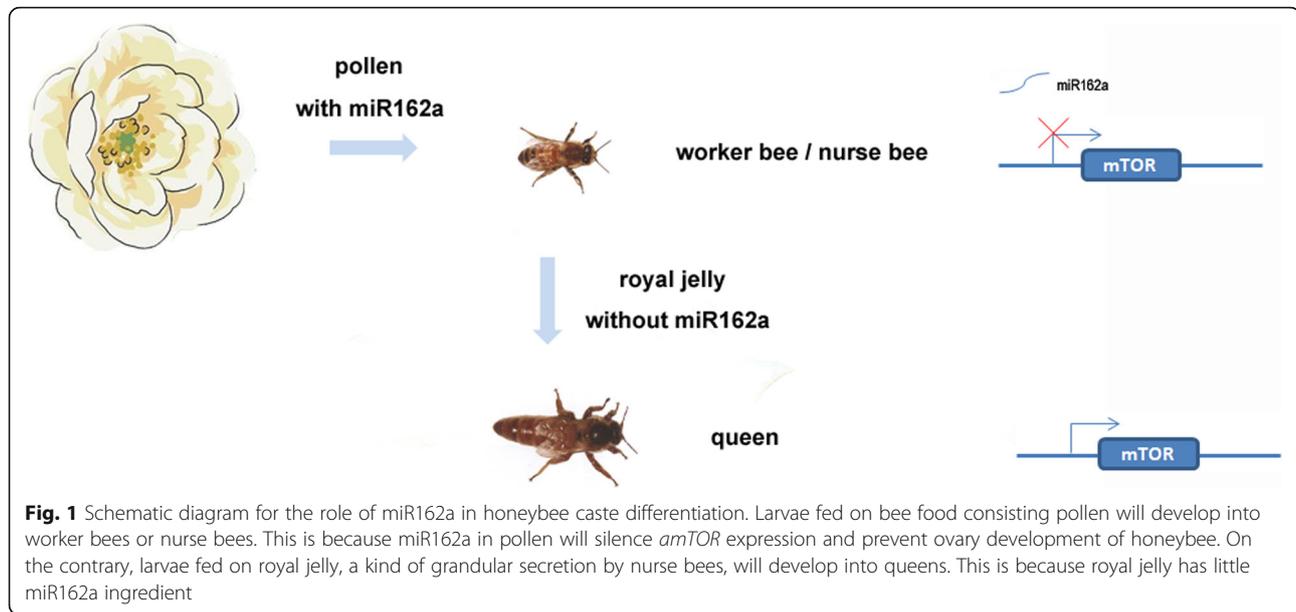
In either plants or animals, miRNA should function in a regulatory network. In this network, how plant miRNA is involved in honeybee Argonaute-complex and how the plant microRNAs are targeted to *amTOR* mRNAs are still unclear. Hence it is also worthy to study the molecular mechanism how plant miRNAs are integrated in honeybee's miRNA machinery. Many studies show that cross-species siRNA transfer comes from interaction between host and parasite [9–13]. In 2012, the authors uncovered that plant miRNAs from food can pass through the gastrointestinal tract into blood and

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plant miRNAs can accumulate in tissues and regulate endogenous gene expression in mammals [14]. Here in this finding, how plant miRNAs enter into and be transferred within bee's body becomes an intriguing question.

However, plant miRNAs may not function alone to determine the caste of bees. A variety of other factors including proteins, sugars, phytohormones, fatty acids, coumarins could also play important functions for honeybee caste formation [1–4]. Coumarins and plant miRNAs are thought to be relatively stable in the wild environment, and there is a crucial methylation step of miRNA molecules in plant miRNA biogenesis [15]. Certain proteins ingredients are less stable as they are easily degraded or denatured. Also some phytohormones can be easily degraded by bacteria [16], and reducing sugar molecules and unsaturated fatty acids are both susceptible to oxidative environment. Overall, it is possible that these factors including plant miRNAs function in a combinatorial manner. Even if plant miRNAs are relatively stable in wild environment, removing plant miRNAs is not sufficient to disrupt the development of all phenotypes related with caste differentiation. Also, plant miRNAs cannot completely reverse the developmental fate like turn worker bees into queens or vice versa [8].

On the other hand, miRNAs may also affect the development of certain flowers by increasing the size of flowers, making a flower more colorful or fragrant to attract more insects [17]. Therefore, it provides a promising clue to indicate plant and insect co-evolution. It is also an interesting question that through which mechanism miR162a functions in controlling flower size or color. In a broad view, how the cross-talk between plant and honeybees happens is also a mystery. For this

cross-talk, the authors raised a hypothesis that when honeybees gather pollen, they pollinate plants; meanwhile, plants donate miRNAs to stabilize the entire colony of honeybees. Hence, there are selective pressures between plants and honeybees on each other in a co-evolutionary relationship. By contrast, for plants which rely on wind to spread their pollen, those plants may not evolve traits to attract honeybees and their poor-quality pollen may affect bee health [18]. It is interesting to test if pollen from anemophilous plants has less miRNAs which can regulate honeybee development. To further uncover the mechanism of co-evolution between honeybees and plants, close collaborations between molecular biologists and ecologists would be a good solution.

In natural condition, plants may undergo biotic or abiotic stress, which may possibly lead to fluctuation of various miRNAs in pollen. The fluctuation may lead to changes of various plant miRNA levels in honeybee food source. Consequently, this will cause negative impacts on honeybee population and caste stability and may provide a possible explanation on a mysterious trend on death of wild honeybee within these decades [19, 20]. Therefore, this finding could also provide new strategies to agriculture to increase the yield of entomophilous crops. In addition, according to the original data sets from this finding, profiles of miRNAs varies between pollens from cole and camellia, especially the content of miR162a [8]. It is also highly possible that profiles of miRNAs from pollens of a certain plant species could vary among different regions. Therefore it is worthy to examine how miR162a widely expressed in pollens of different plant species varies among different regions

where honeybees live. In other words, it would be informative to explore the fluctuation of pollen miRNA ingredients under biotic or abiotic stresses from multiple plant species in different regions to investigate the natural situation of pollen collected by honeybees.

For domesticated bees, nowadays bee keepers are used to feed honeybees with artificial pollen substitutes as bee bread, which is made by wheat, lentil and soy protein, but scarce of plant miRNAs. These protein ingredients are nutritious for honeybee colonies temporarily. However, long-term use of artificial bee bread will compromise the ability of plant miRNA to fine-tune development of honeybees. Studies also suggest that artificial pollens are unable to enhance bee's immunity against parasite [21]. Therefore, researchers should timely advise bee keepers not to feed honeybees by using artificial bee bread alone. On the other hand, as potential solutions to enhance agricultural yields of entomophilous crops, farmers should avoid using artificial bee food as only food source. Instead, they should be encouraged to let honeybees collect food from natural environment. As well, strategies and efforts should be made by local governments to well protect natural biodiversity.

Based on this study, people may potentially worry about that if miRNAs from plants could have negative effects on human health. Although one report shows that certain miRNAs from rice could regulate endogenous gene expression in mammals [14], it does not necessarily mean that pollen miRNAs are harmful to human. Pollen products are not indispensable daily diets, and the plant miRNA level in human body could be too little to cause any physiological effects. Neither there is a *amTOR* homolog in human, which can be targeted by plant miRNAs.

## Conclusion

Overall, in this study Zhu et al. not only provided a detailed study on how plant miRNAs affect honeybee development and caste formation, but also their study will shed light on researches of co-evolution between insects and plants, which has a promising potential in enhancing agricultural yields.

## Abbreviations

miRNAs: microRNAs; siRNA: small interfering RNA

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## Ethics approval and consent to participate

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