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EDITORIAL

What if you can control whom your queen mates with?

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For a very long time honeybee researchers had a monopoly on controlled matings; only they had the means to ensure their queens mated with the guys they wanted her to mate with. That may all be about to change, as Bueno et al. in this issue describe a method that, when refined, might allow controlled matings of stingless bee queens.

There are many reasons why researchers want to control the matings of their queens. It allows neat experimentation, particularly when interested in the inheritance of particular traits (Yagound et al. 2020), or when trying to find out if there are any parent-of-origin effects (Oldroyd et al. 2014; Remnant et al. 2016), to give just a few examples. And of course control over mating facilitates selection for particular traits that might be of commercial value (Evans and Spivak 2010). The invention of artificial mating in honeybees (*Apis mellifera*), perfected first by Laidlaw (1977), was a game changer as now semen could be collected from drones from a particular genetic background and used to inseminate queens. Normally honeybee queens are extremely polygamous, sometimes mating with up to 50 drones while in flight. No wonder bee breeders may want to have some form of control….

Most stingless bee queens are monandrous, but that doesn't mean it is easy to control which male they mate with. Because they can only mate once, one would think that the queens should be pretty picky when it comes to choosing their one and only mate (Smith 2020). As a matter of fact, we don't know that much about the mating behaviour of stingless bees in general, other than that males tend to gather in front of colonies that seem to be rearing virgin queens in so-called mating aggregations (Bueno et al 2023). How the males find out that there is a virgin queen interested in mating is a mystery in itself, as most stingless bee colonies always have a few spare virgins often hiding around waiting patiently to earn the throne in case of the queen's death or when her productivity is in decline (Imperatriz-Fonseca amd Zucchi 1995). In addition, in many species (i.e. *Melipona* genus) it is difficult to distinguish between cells that contain a queen as they are of the same size as cells in which workers are raised (Imperatriz-Fonseca and Zucchi 1995). And because all stingless bees are mass provisioners, meaning that all food the larva requires to reach maturity is placed in the cell before an egg is laid, one cannot easily check the identity of the pupae. All that means that even control over queen rearing in stingless bees is not necessarily straightforward.

Bueno et al. decided to study some of the natural history gaps of a popular Australian stingless bee queen species, *Tetragonula carbonaria,* particularly those related to the life cycle of queens. They tackled both the rearing of queens, or actually their maturation, and their mating in the same study by isolating cells that contained queens (queen-cells of *T. carbonaria* are larger than worker-cells and therefore easy to identify) and monitoring them until they emerged. This way they took the control over which queens are allowed to emerge away from the workers. It also allowed them to monitor the maturation of the queens once they had emerged by placing them into little colonies, closed to the outside and provided with sufficient resources. To find out when the queens were ready to mate, for which they have to leave the colony, Bueno and colleagues put a small plastic tube in the entrance to the box. When the queen was seen entering the tube, she was deemed old enough to mate.

Artificial insemination in honeybees requires the queens to be narcotized with CO_2 so that they do not wiggle around while trying to inseminate them. It also reduces the stress on the queens, and so Bueno et al. decided to include a CO_2 treatment in their experiment. Once queens were seen entering the plastic tube, signalling their readiness to mate, they were inserted, head first, into the cut tip of a plastic pipette. She was then exposed to a naturally-occurring drone swarm so that a male could land on her and initiate mating. One set of queens was narcotized while the other wasn't. Interestingly, none of the CO_2 treated queens had sperm in their

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spermatheca even though males did mate with the immobilised queens. This suggests that the queen actively directs sperm into her spermatheca. And that begs the question if naturally-mating queens exert some control over which male's sperm is allowed into her spematheca. Female choice by stingless bee queens? Perhaps a little far-fetched, but weirder things have been found in nature. Stingless bee queens may be far pickier than what we expected!

Clearly Bueno et al.'s study is only the beginning, but it is a promising beginning. A step forward towards unveiling the mysterious lives of stingless bee queens. As this issue makes clear, stingless bees research is certainly taking off and being able to manipulate the mating of queens will open up a range of new avenues.

Data availability There are no data.

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