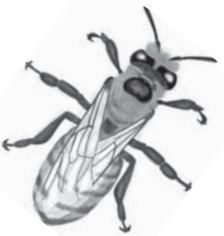
FOUR

Bee Love



Question 1: How does a bee attract a mate?

Answer: Queen honey bees give off pheromones that attract males (drones) when they are ready to embark on their mating flights (see this chapter, question 3: How do bees mate?). Since the sole task of drones is to mate with virgin queen bees, it is unsurprising that drones have receptors on their antennae that are specially attuned to detect queen pheromone. When a virgin queen is ready to mate, she makes several flights to an area where drones from local colonies congregate and wait for a queen with whom they can mate. When the drones become aware of her presence, they compete to mate with her and some of the strongest and most agile succeed.

In solitary species, the males normally emerge before the females, and they may wait for a female to emerge at the nest site or near a flower and then mate with her there. Among bumblebees and some solitary species, the male is responsible for attracting a mate, and he marks a series of plant stems with pheromones that he secretes from glands on his mandibles (jaws). In some species, males are territorial and patrol and guard a certain route, marking it with pheromones. Sometimes other males are attracted as well, and when a receptive female is drawn to the territory, the male or the group of males attempts to mate with her, and in some cases a large mass of drones surrounding the female will fall to the ground in the midst of trying to mate.

Question 2: Do all bees mate?

Answer: Among female honey bees, it is almost always only the queen that mates. She mates multiple times shortly after she emerges from the pupal stage, storing up enough sperm to keep laying fertilized eggs for the rest of her life. The queen releases a pheromone that suppresses the development of the reproductive systems of the female worker bees. This chemical keeps the workers from becoming reproductively viable, and the queen's eggs and larvae reinforce her message with chemicals that they pass along to the attending workers, signaling that the queen is providing the colony with an adequate supply of new workers. If the queen is removed or if her pheromone level drops, worker eggs can develop, although, because workers are equipped with barbed stings, they cannot mate.

The only role of a drone is to mate with a virgin queen when she goes out on her brief mating flights. Drones do not mate with the queen of their own colony—that queen is the drone's mother. A virgin queen may mate with as many as twenty drones, but the rest of the drones will die without mating. If a drone has not successfully mated after a week or so, the workers will withhold food or he will be driven out of the hive and killed. The reality is that most drones die without ever mating (see chapter 1, question 10: What is the role of the drones?).

Question 3: How do bees mate?

Answer: A virgin honey bee queen mates early in her life, having sex "on the fly" with as many as twenty drones over a period of a few days, and then she never mates again. She produces eggs throughout her life in well-developed ovaries that fill up most of her abdomen, and she can produce hundreds of thousands of offspring in her lifetime from the sperm she stores in those few days.

A few days after the young honey bee queen has emerged from her pupal case, she flies to a so-called drone congregation area, where a large number of fertile males from nearby colo-

nies are assembled, waiting to take advantage of their once-in-a-lifetime opportunity to pass on their genes to any virgin queen that comes along. The drones fly around at a height of twenty or thirty feet above the ground, and they locate a receptive queen by sensing her pheromones and by using all five of their eyes. If they are vigorous enough to catch up with a queen, they may get the opportunity to indulge in what has been described as an "acrobatic orgy."

This vivid description of honey bee mating comes from Mark Winston in his 1987 book, *The Biology of the Honey Bee*. "Mounting and copulating are rapid and spectacular . . . with the drones literally exploding their semen into the genital orifice of the queen." Once contact has been made, the mating generally lasts from one to five seconds. "Within a split second, the drone grasps the queen with all six legs and everts the endophallus into the queen's open sting chamber. At this point the drone becomes paralyzed and flips backward, and ejaculation results from the pressure of the drone's hemolymph as the abdomen contracts. The explosive and sometimes audible ejaculation ruptures the everted endophallus and propels the semen through the queen's sting chamber and into her oviduct" (page 207). The ejaculation separates the drone from the queen, and he dies shortly after mating.

Observers report that they can tell when a queen bee is mating nearby because of the large number of dying drones that drop to the ground, sometimes accompanied by a noise like popcorn popping. The drone's severed genitals may act as a temporary vaginal plug, designed to allow time for the drone's sperm to enter the queen's system, but the queen or a subsequent suitor can dislodge the plug, so no drone is guaranteed exclusivity. The queen receives an average of six million sperm from each male, but sperm die in large numbers as they make their way through the female's reproductive tract, and some may be ejected during the course of a subsequent mating. Stored sperm may even be digested in lieu of food in times of famine, so, typically, the queen will retain only about six million sperm from this mass mating to fertilize her eggs. In fact, this multiple mating (*polyandry*)

assures genetic diversity that confers multiple benefits on the colony (see this chapter, question 4: Why does a queen mate with more than one drone?).

In some solitary species of the genera *Nomadopsis* and *Perdita,* the male may remain coupled with the female while she forages or flies back to the nest after mating, preventing other males from mating with her. *Centris adani* males deposit pheromones on the female during mating that repel other males. Male bees in these species also die shortly after mating, and the female begins searching for a place to build a nest. Some solitary females only mate shortly after they emerge from the pupal stage.

Question 4: Why does a queen mate with more than one drone?

Answer: Mating with more than one drone (up to twenty in the case of the honey bee queen) results in a genetically diverse colony, and scientists are discovering the benefits that result from the diversity, at least in the short term. The benefits of genetic diversity in the long-term are difficult to establish because, essentially, only the queen reproduces.

Using instrumental insemination (see this chapter, question 10: Can bees be artificially inseminated?) to create colonies that have been fathered by only one drone and comparing them to colonies where the queen was fertilized by mixed semen from several drones, Julia Jones and her colleagues at the University of Sydney found that the diverse groups were able to keep the temperature in their nests more stable than the genetically uniform colonies because bees from different lineages started fanning at slightly different temperatures, while bees in the homogenous colony all started fanning at the same time.

Other evidence of the adaptive value of genetic diversity was explored by Cornell University scientist Thomas Seeley in a study with Heather Mattila and in another with David Tarpy. Both studies compared genetically diverse colonies, where the queen had been instrumentally inseminated by sperm from ten or fifteen drones, to genetically uniform colonies, where the

queen had been inseminated by sperm from only one drone. The genetically diverse colonies proved to be more resistant to bacteria, built honeycomb at a 30 percent faster rate than homogenous colonies, collected 39 percent more nectar and pollen, and after two months had five times the population of the single father colony. Swarms from diverse colonies also founded new colonies faster than swarms from genetically uniform colonies, another valuable attribute.

Paul Schmid-Hempel and Boris Baer in Zürich compared queen bumblebees that were instrumentally inseminated with sperm from the same drone with others who were inseminated with a mixture of sperm from four drones. The queens then founded colonies in a meadow near Basel, and their progress was tracked. The multi-father colonies were healthier, suffered from much less parasitism than the single-father colonies, and were twice as prolific, further confirmation of the benefits of genetic diversity.

Question 5: How many eggs does a honey bee queen lay in a day?

Answer: A honey bee queen can lay fifteen hundred to three thousand eggs on a good day, and she can lay as many as half a million eggs in her two- or three-year lifetime. Her eggs are only reared to adulthood if there are enough workers to feed and incubate them.

Question 6: How is the sex of a bee determined?

Answer: If a queen lays a fertilized egg, it will become a female worker or, potentially, a queen; if she lays an unfertilized egg, it will become a male. Each bee egg develops in one of a pair of small tubes (*ovarioles*) that make up the queen's ovaries, and once the egg is fully formed, it moves through the oviducts into a tubular passage (the *lumen*). A lifetime supply of sperm is stored by the queen in a little globular sac (the *spermatheca*) in her genital tract, and the queen controls the release of the

sperm, enabling her to choose to lay an unfertilized egg or one that has been fertilized. When drones are needed in the colony, she will lay some unfertilized eggs. This peculiar system of reproduction is known as *haplodiploidy* because the drones are *haploid,* meaning they have half the normal chromosome content, and the queens are *diploid*—with a full set of chromosomes—like most animals.

But Soochin Cho and colleagues established that it is not quite that simple. In humans, sex is determined by the combination of sex-determining chromosomes derived from both parent's sets of genes (XX for females, XY for males). In the honey bee, specific combinations of different versions (alleles) of a sex-determining gene determine the sex of the offspring. If the bee has two different alleles, the sex-determining gene will be female; if it has only a single version of the gene, it will become a normal, fertile male. But if the queen has mated with a male who has a version of the sex-determining gene that is identical to hers, the fertilized eggs produced from his sperm that have two identical sex-determining genes will yield sterile male offspring, and these drones will be eaten by females in the colony since they cannot reproduce and therefore serve no purpose. Multiple matings reduce the proportion of sterile males that will be produced because not all mates will have a matching sex-determining gene. Jay Evans of the U.S. Department of Agriculture and colleagues have written a clear review of this sex-determining mechanism that was first identified by Martin Beye and others.

Question 7: What is royal jelly and how does it produce a queen?

Answer: Some people believe that the bitter-tasting, nontoxic royal jelly is a healthful component of the human diet, but scientific studies do not support that belief. But for a female honey bee, it makes the difference between developing into a queen or becoming an ordinary worker. A fertile queen and a sterile

worker have the same genetic makeup (genotype), but they have very different traits (phenotype). The queen bee is large, mates and lays thousands of eggs, and can live for several years; a typical female worker bee has a reproductive system that never develops, is considerably smaller than the queen, and generally lives only several weeks. These differences occur, as the saying goes, because "you are what you eat."

Instead of being fed the usual brood food and bee bread, like a larva that is destined to become an ordinary worker (see chapter 2, question 2: What do larvae eat?), a larva that will become a queen is fed a substance, called royal jelly, that has a different chemical composition. Any female larva can become a queen if she is cared for properly by being fed royal jelly during the early stage of larval life. In the bee, the royal jelly causes a group of genes to be activated differently than they would be on a worker's diet, resulting in significant physical and behavioral differences (see sidebar on epigenetics in chapter 3). Hormones also function differently in the queen than in an ordinary female worker.

When the worker bees sense that the colony's resident queen is failing, they respond by creating some larger-than-usual cells, called queen cups, in the brood comb and encouraging her to lay eggs in them, so they can begin to rear a few new queens. A new queen must quickly supercede or replace the old queen if the colony is to survive. The worker bees produce royal jelly from a specialized gland (the *hypopharyngeal* gland) in the head, and they deposit a steady supply of it into the especially large cell where the queen larva is developing. Royal jelly is a thick, milky fluid with the consistency of plain yogurt, and it contains more protein and sugar than the food given to worker bee larvae. Royal jelly consists of approximately 12 percent sugar compared to about 4 percent in worker larvae food, and the queen larva is visited by nurse bees approximately 1,600 times, compared to 150 visits to a worker larva per day. These qualitative and quantitative differences produce dramatic results, and by the end of the larval stage, the queen is larger and heavier, she has a higher

metabolic rate, and she has fifteen times the level of growth-stimulating juvenile hormone than that found in worker larvae at that stage.

Royal jelly also contains *vitellogenin,* an egg yolk precursor whose synthesis was investigated by Preeyada Koywiwattrakul, Graham Thompson, Sririporn Sitthipraneed, Benjamin Oldroyd, and Ryzard Maleszka. The activity of the vitellogenin gene was found to be diminished or stimulated depending on the development of the ovaries. When groups of caged, queenless worker bees were treated with carbon dioxide (as is used as temporary anesthesia for artificial insemination of honey bee queens), they showed low levels of ovarian development as compared to controls that were not given the carbon dioxide treatment. The bees with inhibited ovaries had lower levels of expression of the vitellogenin gene. This finding may be of interest in understanding the regulation of sterility in worker bees. A queen's ovaries do not become completely activated until she mates, so when a virgin queen has been instrumentally inseminated, she is temporarily anesthetized with carbon dioxide, which stimulates her ovaries (see this chapter, question 10: Can bees be artificially inseminated?).

Question 8: How is the queen bee chosen?

Answer: There is no election process to become the new queen: she is not chosen, but presents the best traits among the queens that emerge. Once the old queen has swarmed (see chapter 8, question 2: What is swarming?), the first virgin queen that emerges from her cell is soon ready to take over as the reproductive focus of the colony. She begins signaling her presence with audible vibrations (see chapter 3, question 8: What is piping behavior?), which sometimes are enough to prevent any other virgin queens from emerging. This queen may also kill other queens in their cells before they can emerge. If another virgin queen manages to emerge, there may be a fight to the death between the queens. The departing queen is guided by

a group of as many as ten or twenty thousand worker bees in a primary swarm. If more than one new queen emerges in the old colony and the first to emerge does not kill the others, there may be subsequent smaller "after" swarms, each led by a new queen.

Question 9: Can bees of one species mate with another species?

Answer: Honey bees cannot mate with other types of bees, like bumblebees or carpenter bees, but all of the breeds of *Apis mellifera,* the European honey bee, can interbreed. And beekeepers can deliberately produce hybrids to improve disease

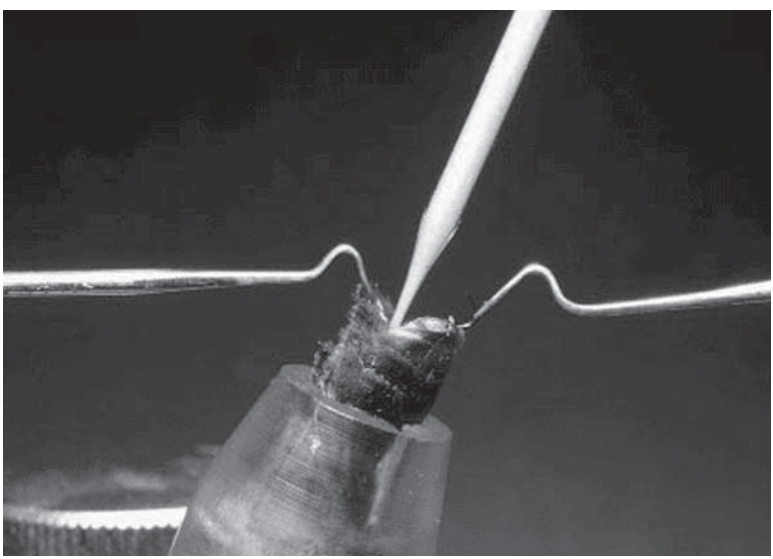


Fig. 17. With temporary anesthesia, honey bee queens can be instrumentally inseminated with drone semen using a laboratory apparatus. This close-up photo shows the process in detail. The queen's body is held in a small tube. While small hooks hold her abdomen open, a syringe deposits semen into her reproductive tract in a structure called the bursa (see chapter 4, question 10). *(Photo by Sue Cobey.)*

resistance and honey production and to create more prolific, gentle strains. Some of the advantages in the initial generations of deliberately created hybrids may be lost as subsequent generations crossbreed naturally. Some hybrids may become unacceptably aggressive or may have developmental defects.

Question 10: Can bees be artificially inseminated?

Answer: Artificial insemination of bees, known by beekeepers as *instrumental insemination,* was first demonstrated in 1927 after over one hundred years of failed attempts using all sorts of imaginative techniques. Beekeepers are always trying to improve their stock by developing bees that are more disease resistant, better honey producers, and more docile. As the natural survival of honey bees becomes more difficult (see chapter 10, question 10: What is colony collapse disorder?), insemination is being used for research and breeding experiments to try to uncover the causes of the decline. Researchers also use this technique to create colonies with certain characteristics that they are interested in studying (see this chapter, question 4: Why does a queen mate with more than one drone?).

In the 1940s it was discovered that multiple mating is normal for queen bees, so when a queen is inseminated, semen is usually collected from many drones and it is combined in a syringe. The age of the virgin queens and drones at the time of insemination is important because they must be sexually mature. Queens need to be from six to nine days old, and drones should be at least sixteen days old. Collecting a drone's semen may be accomplished by holding him by the head and thorax and stimulating the abdomen. It may be necessary to apply gentle pressure to the tip of the abdomen in order for the endophallus (internal genital tract) to be everted so that the semen can be exposed and collected. If this approach is not successful, the head and thorax of the drone must be crushed and the endophallus forced out by firmer pressure on the abdomen.

Fig. 18. A queen mating yard in Florida, with many small hives created to house new queens. Other nearby colonies rear drones to ensure that the virgin queens have ample mates. *(Photo by Gerry W. Hayes Jr.)*

The queen is placed in a holding tube and anesthetized with carbon dioxide, and the semen is injected into her reproductive tract. It may seem cruel that some drones are killed in the collection process, but since they die soon after mating naturally, their loss does not cause negative consequences to the colony (see this chapter, question 3: How do bees mate?).