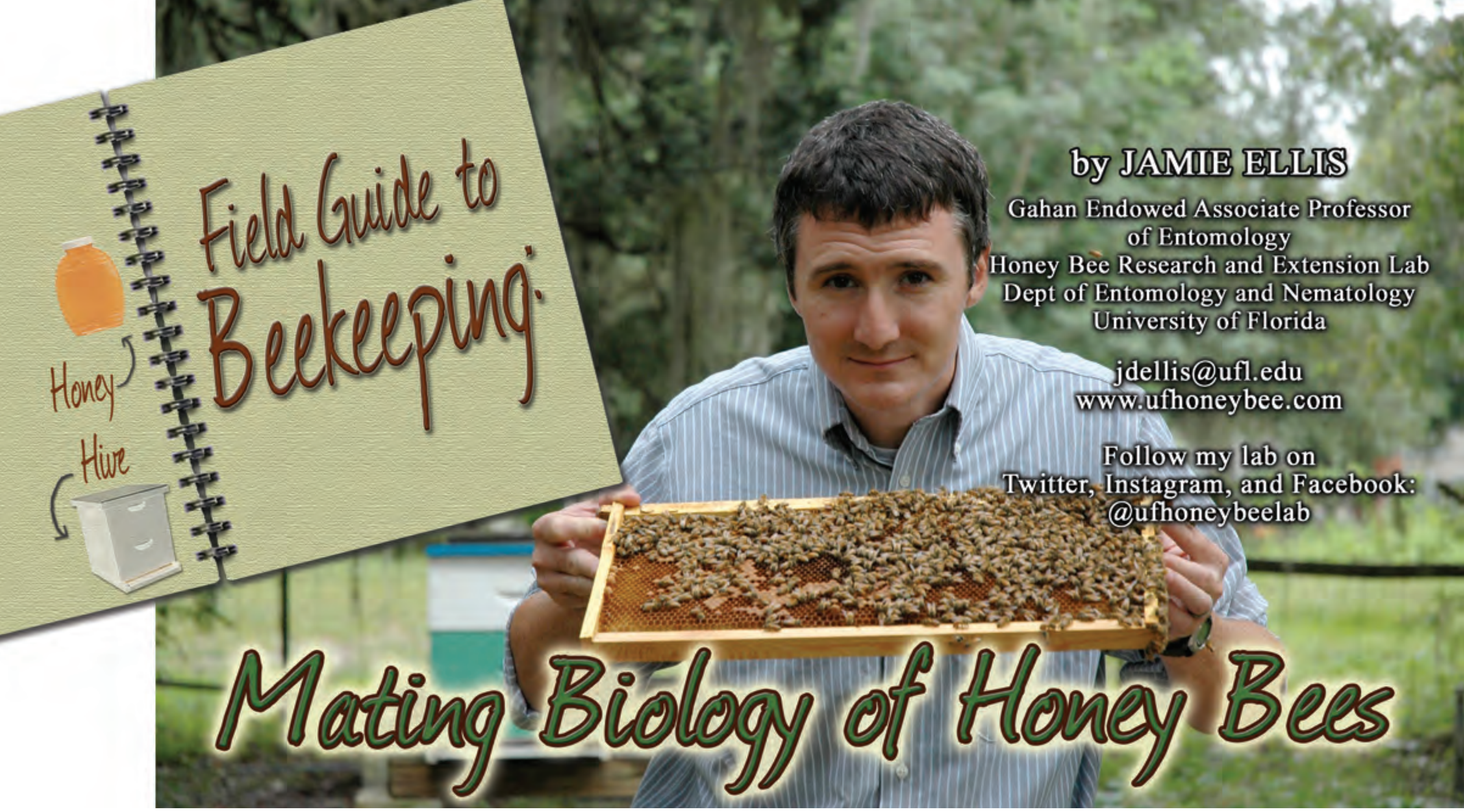

Mating Biology of Honey Bees

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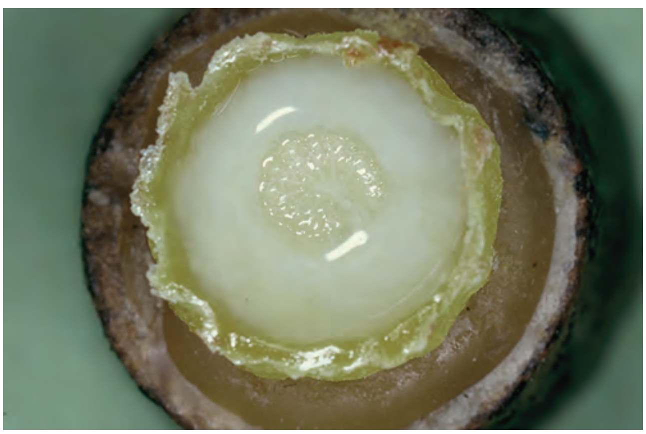
A few years ago, I had the distinct pleasure of meeting Drs. Niko and Gudrun Koeniger. Dr. Niko Koeniger is the former head of the German bee research institute in Oberursel, Germany and professor of biology at the Goethe University in Frankfurt. Dr. Gudrun Koeniger worked as a research scientist and editor of the international bee journal *Apidologie*. Both have devoted a lifetime to the study of honey bee diversity and biology. Among other things, they worked tirelessly to decipher the intricacies of honey bee mating biology. Their work culminated in a book on the topic written for German beekeepers. The book is entitled (English translation) *Mating Biology and Mating Control*.

In 2014, Niko and Gudrun approached me about producing a revised English version of their German book. Thus, work commenced on *Mating Biology of Honey Bees (Apis mellifera)*, a book we coauthored with Dr. Larry Connor of Wicwas Press. Much of what I share in this article is taken from our joint book. (Caution: shameless self-promotion follows.) Feel free to read the book to discover more information on the mating biology of honey bees if this topic is of interest to you. The full citation for the book can be found in the reference section at the end of the article.

Rearing and sexual maturation of queen honey bees

Typically, honey bee colonies are headed by a single queen whose primary responsibility is to produce offspring (Ellis, 2015c). There are times in the normal lifecycle of a colony when a new queen must be produced. This occurs when a colony swarms (Ellis, 2015a) or when a queen is lost for whatever reason (death, maimed, etc.). I will not spend any time in this section discussing what leads to a colony's decision to make a new queen, but rather I will discuss queen development from egg to sexual maturity.

Queen honey bees result from fertilized eggs that are destined to become female, but not otherwise predetermined to be either a queen or a worker. The decision to make a female larvae a queen or worker lies with a colony's worker bees. Worker honey bees in the nest take a female larva that emerges from a fertilized egg and direct her to roy-



(l) Figure 1. A queen larva floating in a pool of royal jelly. The queen cell remains open (uncapped) as the immature queen develops to adulthood in her cell. This photograph was taken from the bottom of the queen cell. In nature, the cell would be turned such that the opening would face down, with the queen developing suspended from the royal jelly above her. ***Photograph provided by Niko and Gudrun Koeniger.*** **(r) Figure 2. A queen bee emerging from a queen cell. This photograph is oriented correctly, showing the queen emerging upside down from her royal chamber.** ***Photograph provided by Niko and Gudrun Koeniger.***

Figure 3. Queen cells on the bottom of a brood comb. A queen emerged normally from the cell on the left, this evident by the fact that the cell is opened at its tip. The newly emerged queen then proceeded to bite holes in the neighboring queen cells, insert her abdomen in the cells, and sting her competition to death. Worker bees further widened the holes in the cells and removed the lifeless contents. *Photograph by Keith S. Delaplane.*

alty by feeding her more and better quality food (royal jelly – Figure 1) than that fed to her sisters who are to become workers. This they can do because the female larvae remain bipotent (can become either queens or workers) up to 48 hours after emerging from their eggs. Caste determination of the female larvae becomes final on the larvae's third day after emergence, at which time the workers begin to offer the developing monarchs copious amounts of royal jelly. This rich food ensures rapid growth of the developing larvae, whose weights increase 600-fold during their first six days of life.

Queen honey bees develop in peanut hull-shaped cells that hang vertically from the face or periphery of the brood comb. This means that the developing queens hang upside down while maturing. It takes a queen about 16 days to develop from egg to adulthood, this likely because of the large volume of good food she receives while young. When she is ready to emerge, the queen will use her powerful mandibles to cut through the cell capping, and emerge upside down into the colony (Figure 2).

Figure 4. The everted drone endophallus. A drone's endophallus is the equivalent to the mammal penis. The endophallus is stored, inside-out, in the drone's abdomen until use. At this time, much of the drone's hemolymph (blood), rushes into the endophallus to force it out of the drone's body and into the queen's sting chamber. *Photograph provided by Niko and Gudrun Koeniger.*

The first emerging queen has hit the jackpot, of sorts. She is first in line to inherit the nest and the colony of bees it contains. If she is successful, she will be fed, groomed, and otherwise pampered daily as she progresses through a life that is many times longer than those of her sister workers and eventual offspring. However, she has some work to do before she can receive her inheritance.

Newly emerged queens seek out and eat the food necessary to power their upcoming activities, the first of which involves getting rid of the competition. As you might imagine, a colony does not usually attempt to rear only one queen when they intend to swarm or otherwise need a queen. Instead, they invest in the production of dozens of queens, all of which are developing alongside the first queen to emerge. The first queen to emerge, consequently, becomes a stealthy assassin who searches for her developing sister queens so that she can eliminate the threat they pose to her impending assentation to the throne.

A newly emerged queen will begin checking all of the brood combs in the nest, doing this at a high rate of speed. She is searching for two things: (1) already emerged queens and (2) queen cells containing yet-to-emerge queens. When she encounters the first threat, she begins to fight the queen until one of them is stung, dies, and is removed from the nest by undertaker workers. During intermittent battles with any adult queens she may find, the new queen turns her attention to her immature sisters developing in queen cells. When she finds queen cells, she will bite a small hole in the side of the cells, insert her abdomen, and sting her helpless sisters to death. These cells, then, are opened from the sides and the lifeless contents removed by the colony's workers. You can always tell when a queen cell has been attacked by a new queen because it will contain a large hole in its side (Figure 3). Virgin queens will repeat these behaviors until all of their competition is eliminated and only one queen remains in the nest.

The surviving queen remains in the hive over the next few days. During this time, she surveys the combs, eats, and continues to mature. She even enjoys long periods of rest and food on demand, provided to her by her ever growing retinue of attendant workers. This time of rest and development is necessary given that she is not sexually mature at emergence. She needs more time for her flight muscles, ovaries and spermatheca to develop. The latter play an important role in queen reproductive biology and were reviewed in my September 2015 ABJ article (Ellis, 2015b). It will be useful to refer back to this article once I begin to discuss mating biology below because some of the discussion of what happens inside the queen after mating will get a bit technical.

The newly emerged queen is quite active. She will run through the nest, a behavior that may even be encouraged by the workers who are increasingly aggressive toward her around the second or third day. Around this time, worker bees nip (bite) at the young queen, thus encouraging her to run throughout the colony. Also during this time, the queen is likely to encounter any queen cells or emerged queens that she failed to find during her first colony search. The queen reaches sexual maturation around 22 days after being laid as an egg (6 or so days after emerging from a queen cell). A queen's behaviors change considerably once she reaches maturity.

Rearing and sexual maturation of drone honey bees

Drones are the other reproductives in the honey bee colony. They are not participants in the social activities undertaken in the nest. Rather, their sole purpose is to mate with a young queen. Consequently, they are valued during the reproductive season, but are otherwise viewed as useless during the fall/winter when no new queens are being produced. This truth is best demonstrated by the late season phenomenon that occurs when worker bees force drones out of the nest so as not to waste resources to maintain a group of bees that will not help the colony survive winter.

Worker bees in the nest typically permit the production of drones immediately prior to and throughout the season that queens are produced, i.e. during the mating season. Drones mature from egg to adult in about 24 days, hence a colony's need to begin drone production in advance of queen production.

Drone honey bees result from a queen's conscious decision to lay an unfertilized egg in a large brood cell (a drone cell). Queens inspect cells prior to laying in them. During this time, she will insert her head and front legs into the cell, the latter which she uses to measure the cell's interior diameter. This way, the queen is able to determine if the cell is small or large, thus knowing whether to lay a fertilized egg that will result in a worker or an unfertilized one that will result in a drone.

Interestingly, the worker bees are the primary regulators of the number of drones in a colony. They do this by controlling the number of drone cells that they develop and are available for the queen to use, allowing only about 13-17% of all cells available in the nest to be drone cells. They also will cannibalize immature drones when foraging conditions are bad, the colony is stressed, etc. Queen bees can contribute to this regulation by controlling the number of fertilized eggs that they lay. Queens usually do not lay in drone cells later in the season (summer/fall) or when environmental conditions are poor.

All of this produces an interesting anomaly in colonies. Typically, colonies rear about two times more drone brood than what ultimately becomes adult drones in the nest. For example, the average colony works to rear about 5,000 immature drones (drones in the egg/larva/pupa stages) per season, but only about 2,500 of these reach adulthood. Thus, there seems to be a high loss of drones during their development, a phenomenon likely closely controlled by workers. Only about 5% of the bees in the nest during reproduction season are drones.

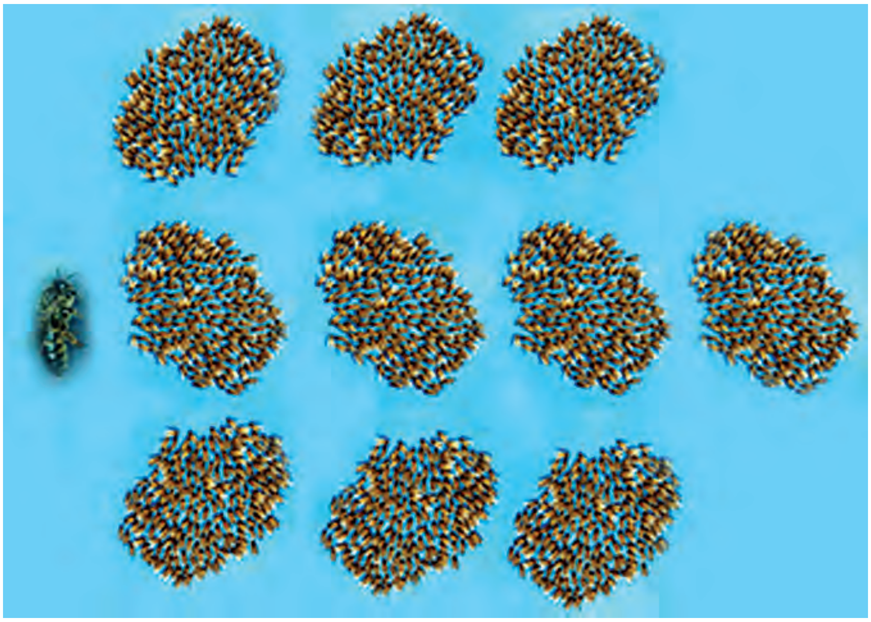


Figure 5. An illustrative view of the male-biased ratio in honey bee reproductives. A colony will make about 1 - 2 adult queens for every 2,000 or so drones. Seen here is a single queen (left) and 10 groups of drones with 200 drones per group (queen and drone size not to scale). ***Photograph provided by Niko and Gudrun Koeniger.***

Drones are fed a food similar to that fed to workers and otherwise develop much the same way, just slower, about 24 days in total. It takes drones another 12 or so days to mature sexually after emerging as adults. Thus, in total, it takes drones about 36 days to go from egg to sexual maturity, while in comparison, it takes a queen about 22 days to do the same.

Sexual maturation in drones is considerably different than that in queens. The testes of a drone are formed in the embryonic drone. Headless/tailless sperm begin to develop in larval drones the moment that they hatch from their eggs. The testes continue to grow in the developing drones as the immature drones are tended and fed by their worker sisters. The sperm cells stop multiplying in drones about six days after the drone hatches from the egg. At this point, the testes are as large as they will ever be. When the drones become pupae, the sperm mature into spermatozoa, including developing a head and a long tail.

The drone endophallus (the drone equivalent of the penis – Figure 4 and see Ellis, 2015b) is stored inverted in the drone's abdomen until time for use. The endophallus begins to develop in the drone around the 7^{th} pupal day. The structure of the endophallus is somewhat complex and it contains various appendages that will be discussed in the mating section of this article. It is mature only upon adult drone emergence from the cell.

A drone's sperm migrates from its testes into seminal vesicles about three or so days after the drone emerges from its cell as an adult. The migrating sperm attach their heads to the cells lining the walls of the vesicles so that they can absorb secretions from the gland cells there, thus completing their development. Most of the sperm have completed their migration from the testes to the seminal vesicles around 12-13 days after drone emergence from the cell. At this point, the testes are completely shriveled while the vesicles are swollen with sperm. The sperm are nourished in the vesicles and remain alive and functional until the drone's death or through their life in a queen, assuming the drone is able to mate with a queen.

A drone will begin flying around 8 days after emerging, but then mainly to defecate. These cleansing flights last about two to three minutes. When drones are about two weeks old, they will remain out of their colonies 25 minutes or longer, looking for queens with which to mate. Drones that fail to mate will live, on average, about three to five weeks, though some have been shown to live 50 days.

Interestingly, colonies produce considerably more drones than they do queens. The typical colony will produce 1-2 adult virgin queens for every 2,500 or so sexually mature drones per season (Figure 5). This is a sex ratio between 1:1,250 and 1:2,500 (male:female) when sex ratios usually are closer to 1:1. This is quite a male-biased sex ratio and leads one to question why is it so much cheaper for a colony to produce drones than it is to produce queens. The answer is simple but possibly not so intuitive unless considered carefully. A colony's investment in a new queen, and in her success, lasts the entire life of the pampered queen, far beyond that required for the short lives of even 2,000 drones that normally operate alone, with little input needed from workers. Thus, the successful rearing and maintenance of a mature, productive queen seems to be as costly to the colony as rearing thousands of drones, most of which will never fulfill their lives' purpose.

The mating flights of queens and drones

Now that the queens and drones are sexually mature, they are ready to mate. Honey bee queens and drones do not mate in their nest. The main reason for this is that the resulting queen is related to all of the drones available in the nest. Instead, sexually mature queens and drones leave their colonies daily in search of members of the opposite sex with which they can copulate. These flights, called "mating flights," occur at very specific times and locations for queens and drones, mainly in an effort to maximize the mating success of both sexes. Keep in mind, leaving the nest is risky, especially for queens. It was reported in a few manuscripts that 6 - 26.5% of queens are lost on mating flights. Clearly, there must be a benefit to colonies for queens to mate in the air, outside of the hive, if the queens are willing to take such a risk. There is a benefit: inbreeding is minimized.

The new queen is ready to take her first mating flight when she is between 6 and 10 days old. Her first flights are usually short, averaging less than five minutes per flight. These flights are believed to be orientation flights that the queen takes to learn the location of the nest and nest entrance. After learning where home is, she will leave for mating flights that last 15-30 minutes. Most queen mating flights occur between 2:30 – 4:00 pm. This time period can fluctuate a bit depending on where the colonies are located. Regardless, the total flight period for potential queen mating flights at any given location is about 1.5 hours. Queens will spend about 30 minutes per day for 1-2 days on mating flights.

In contrast, drones begin their search for mates when they are about 12 days old. They usually leave the colony about 1:00 pm with peak drone flight happening between 2:00 – 5:00 pm. Thus, the three hour period over which they conduct their mating flights is about twice that of the average queen. Given that drones spend about 25 minutes on a mating flight and can do this over a period of three hours, the average drone can make about six mating flights per day in search of a queen. They will do this about the last two weeks of their adult lives.

It is important to note that weather conditions are the main factors influencing the mating flights of both sexes. Queens prefer to fly when temperatures are above 68°F (around 20ºC) and when the skies are clear or partly cloudy. Drones will begin flying at about 64°F (about 18ºC) but otherwise prefer similar flight conditions to those preferred by queens. Both sexes can start their flights in less favorable conditions, but they usually return to the colony within one minute of leaving the nest if the conditions are less than ideal.

As a quick aside: queens can begin their mating flights as late as three or four weeks after emerging from their cells. However, this typically happens only when the weather conditions otherwise prohibit earlier flights. Queens waiting too long to take their mating flights may fail to mate altogether, this because they missed their window of opportunity. In this case, and after about 30-40 days, the queen will begin to lay unfertilized eggs that result in drones. The occurrence of bad weather when queens should be mating is one of the causes of drone laying queens, or "drone layers" as beekeepers often call them.

Where do queens and drones go when they leave the nest? This was the subject of considerable research over the last 50 years. It turns out that queens and drones both go to areas known as drone congregation areas (or DCAs). As the name implies, DCAs are locations where the area's drones from various colonies congregate to wait for the arrival of young queens.

Drone congregation areas are studied using drone traps. A drone trap is a conical mesh cage that has been tethered to a string and is suspended in the air by a helium-filled weather balloon (Figure 6). The trap contains multiple dummy queens, or small pieces of black rubber or cotton to which a synthetic queen pheromone (9-ODA) has been added. From a distance, drones are attracted to the smell of the pheromone. Up close, the drones use their large eyes to orient visually to the queen dummies. In their pursuit of the queen dummies mounted at the bottom of and in the trap, the drones fly up into the trap and are captured for study.

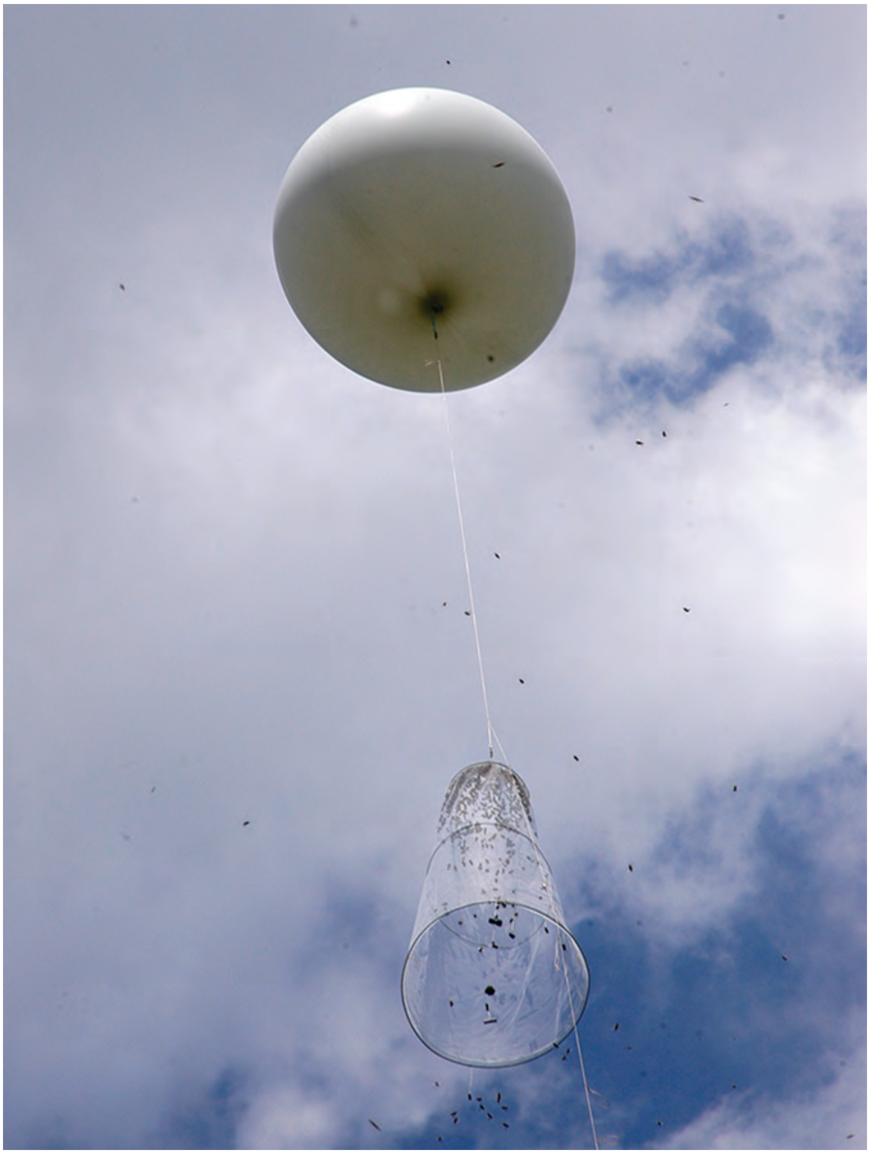


Figure 6. A drone trap. A drone trap is a conical mesh cage (lower 1/3 of the photograph) that has been tethered to a string and is suspended in the air by a weather balloon (upper 1/3 of the photograph). The trap contains multiple dummy queens, or small pieces of black rubber or cotton to which queen pheromones have been added. Drones are attracted to the traps because of the pheromone, and then visually orient to what they believe to be queens up in the trap. This way, the drones will form a small cloud (a drone comet) around the trap, and self-collect in the trap during their search for the queen. *Photograph provided by Niko and Gudrun Koeniger.*

Drone traps can be used to find DCAs. Simply walk around an area with a drone trap in tow. You know you have found a DCA when you start getting large aggregations of drones around the suspended cage.

Using drone traps, scientists discovered quite a bit about DCAs. For example, DCAs often are situated in the direction of what the Koenigers call "depletions of the horizon." Imagine, for example, standing in a valley between two rows of mountains or hills. Looking down the valley, with the hills on either side, produces the appearance of a V-shaped horizon in the distance – where the mountains or hills on both sides seem to come together. This is a "depletion of the horizon." Such depletions do not occur as often in flat places such as fields or in the entire state of Florida. So, drones there tend to form DCAs near forest edges or other similar areas showing significant geographical changes. DCAs located in hilly or mountainous regions seem to be better defined than those located in flatter areas where fields are more common.

Using drone traps, investigators also were able to determine that the DCAs in an area tend to remain stable from year to year. This means that this year's drones are flying to the same DCAs that last year's drones, drones 5 years ago, and even drones 5 years from now will use, pending no major environmental changes occur. This shows how important the characteristics of a "good" DCA are to drones and how to search for and recognize these characteristics seems inherent in every drone. That said, we are far from understanding how drones from a given area choose a DCA, what makes a "good" location, and how drones can use them from year to year. This, no doubt, will be the subject of quality research in the future.

DCAs occur about 30 – 80 feet in the air and around 15,000 (9,000 – 26,000 have been found) drones aggregate in these areas at one time (daunting odds for any would-be queen suitor). The height of DCAs from the ground may vary by honey bee subspecies, as has been suggested by some scientists. Though there can be multiple DCAs in an area, drones typically prefer to go to the DCA that is closest to their hive. In contrast, queens usually prefer to visit DCAs further away from the hive, likely in an effort to avoid inbreeding.

Figure 7. A drone comment. Queen honey bees flying through a DCA will attract thousands of drones that fly in unison to chase her. This cloud of drones is referred to as a drone comment. *Photograph provided by Niko and Gudrun Koeniger.*

Drone usage of DCAs close to their hive may hinge on their strong desire to mate with a queen. Given this desire, they tend to go to closer DCAs so that they can spend less time flying to/from a DCA and more time searching for queens at a DCA. Based on the amount of honey (fuel) they can hold while flying, drones can spend about 13 minutes at a DCA that is roughly 1 mile away from their hive and about 19 minutes at a DCA about half a mile from their hive. On the other hand, queens need only a few minutes to mate with all the drones with which they need to mate. Thus, they can afford to fly 10 mins to reach a DCA, spend 5-10 mins at a DCA, and fly 10 mins back. In all, she can visit a DCA that is two miles away in the 25-30 minute adventure.

Reproductive behavior at the DCA

Young queens flying through DCAs attract a large number of drone suitors (Figure 7). This cloud of drones is called a drone comet. The queen is flying fast and, given the number of drones chasing her, any one drone has only a small chance of catching and mating with the queen. Initially, the drone is attracted to the queen by detecting her pheromone, but he finds her when close using his eyes. In fact, his large eyes help the drone hone in on the queen while she is in flight. This is needed because the young queen changes her flight path constantly while in a DCA.

Eventually, one of the drones in the DCA will catch the queen successfully. At this point, he must copulate with her, and docking is not an easy process. After all, the drone usually is positioned behind and below the queen once he catches her. He accelerates to fly above the queen and touches his thorax to her abdomen. The drone uses his first four legs to grip the queen's abdomen from the sides and his last two legs to grip it from below. He holds the queen tightly this way (Figure 8A).

At this point, the drone adjusts his position to align the tip of his abdomen to that of the queen's. The queen, in return, opens her abdomen to receive the drone's endophallus. The drone is able to fly and keep pace with the queen during this complicated aerial maneuver. At this point, the drone will contract his abdominal muscles. As a result, nearly all his hemolymph (blood) is forced into his inverted endophallus, which forces the endophallus out of the drone and into the queen. Having lost much of his hemolymph to his

Figure 8. Copulation between a drone and a queen. The drone catches and mounts the queen in mid-air (A). Next, he inserts his endophallus and his grip on the queen is lost (B). The drone falls backward paralyzed (C). Following this, the queen releases his lifeless body to the ground below. *Photographs provided by Niko and Gudrun Koeniger.*

endophallus, the drone becomes paralyzed and begins to fall backward (Figure 8B).

The endophallus is anchored in the queen via its various anatomical appendages, including a hairy patch on its surface and half-everted horns (the horns can be seen in Figure 4). This has to be a firm anchor, given that the queen must hold her paralyzed partner while flying. Since the drone is paralyzed, the queen has to ensure the transfer of sperm without any help from the drone. She begins to contract her sting chamber and other muscles in her reproductive tract. This pressure causes the drone's endophallus to evert fully. The sperm are released from the endophallus and into oviducts present in the queen's abdomen.

During the final eversion of the endophallus, the drone's body is pushed further backwards (Figure 8C). At this point, the firmer structure of the endophallus begins to peel away from softer structures and glandular secretions surrounding it. Thus, the drone falls down to the ground to die while leaving a part of his endophallus in the queen. This endophallus remnant is known as the "mating sign" or "mating plug" (Figure 9). The entire copulation procedure from catching and mounting the queen to drone release to the ground takes less than two seconds!

The purpose of the mating sign was the subject of much debate and confusion for some time. Many people noticed the mating sign present in queens returning to colonies from their mating flights. This led many people to suppose that queens had to return to the colony between each mating flight. After all, they could not remove the sign from themselves so the workers must have had to do it. However, Gudrun and Niko Koeniger found that this was not the case. Instead, the mating sign plays an important role in the success of other drones' ability to mate with the queen. What the Koenigers were able to show is that the mated, flying queen continues to be receptive to additional drones even though she contains a mating sign. In fact, successive drones wait in a que to mate with her, even while she is copulating actively with another drone (Figure 10). Thus, they found that the next drone with which the queen mates is the one responsible for removing the mating sign. Interesting, they showed that subsequent drones cue into the mating signs left by previous drones in order to mate with the queen more successfully!

Why and how does this happen? First, the Koenigers were able to show that queens containing mating signs from a previous drones were more "attractive" to subsequent drone suitors who could find them easier. In fact, the Koenigers became convinced that the mating sign from one drone serves as a visual cue for the next drone. Next, the Koenigers found that the next drone to mate with the queen, while everting his endophallus into the queen, is able to remove the previous drone's mating sign as part of his docking routine with the queen. Thus, only the last queen's partner leaves a mating sign that must be removed by worker bees in the nest. It appears that the mating sign developed as a means to help subsequent drones mate successfully with the queen. Though this seems hard to comprehend on the front end (after all, do you not lessen your chance of producing offspring if you help your mate copulate with other males?), it does has a reproductive advantage for the drone. It has been shown that colonies headed by queens who mate with a large number of drones are fitter than colonies headed by queens who mate with fewer drones. Thus, it benefits a drone to help his successors because more of his offspring will be born if the queen mates with more males.

Though the mated drone falls to his death on the ground below, the queen continues her mating flight. In fact, the typical queen has been shown to mate with an average of 11 – 17 drones, with a high of 59 being reported. Queens appear to "count" the number of mates to determine when it is time to cease their mating flights, this being how she monitors her own mating success. Perhaps the most interesting thing about this is that *this all occurs during one or two mating flights and over the course of only a few minutes*.

What happens inside the queen after the mating flight is over?

After deciding not to mate with any more drones, the queen returns to the nest, but she is not yet ready to begin laying eggs. She must first process all of the semen that she received from each of the drones with which she mated. For the discussion that follows, it will be helpful to refer to my discussion of the internal anatomy of honey bee queens (Ellis 2015b).

While mating with multiple drones, the queen will receive 70-100 million sperm into her median and lateral oviducts. [As a quick review, the two lateral oviducts come from their respective ovaries and unite to form a single median oviduct. Eggs travel from the ovary that produced them, down their lateral oviduct, and into the median oviduct before passing from the queen's body.] The sperm is not yet moved to the spermatheca. Instead, it resides in the oviducts, awaiting further processing by the queen. Interestingly, the spermatheca can only hold about 5-7 million sperm. So, the queen collects considerably more sperm from drones (10 – 20 times more sperm) than she can store. She must discard the rest of the sperm from her body before she is able to lay eggs. She does this within one to two days of her final mating flight.

The queen must fill her spermatheca before discarding the excess sperm. The queen possesses a series of muscles in her abdomen that, collectively, can be referred to as the "sperm press." The contractions of these muscles push the sperm toward the spermatheca oviduct (an oviduct leading from the spermatheca to the median oviduct). The spermathecal duct has a muscle system as well and these muscles pump sperm into the spermatheca. The filling of the spermatheca this way ensures that the sperm contributions from many drones will make it to the spermatheca. However, it also means that there will be an uneven amount of sperm contributed by each drone represented in the total load in the spermatheca. While there is no "last drone advantage" (i.e. the last drone to mate with the queen does not

(l) Figure 9. The mating sign. The mating sign from the queen's last drone partner can be seen protruding from the tip of her abdomen. Workers in the nest will remove the mating sign from the queen. *Photograph taken and provided by Mike Ledoux.* (r) Figure 10. Even while the queen is mating with one drone, other drones mount and ready themselves to mate with her during flight. *Photograph provided by Niko and Gudrun Koeniger.*

seem to have a greater proportion of sperm represented in the spermatheca), it does appear that sperm location in the oviducts during the one to two day wait plays a role in its likelihood of making it into the spermatheca. Research has shown that despite the number of drones with which a queen mates, only about 8 drones are responsible for siring most of the workers in the nest. The rest of the drones contribute a much smaller percentage to the worker pool. All sperm not making it into the spermatheca (i.e. about 90% of it), is expelled by the queen.

Queen bees are able to keep the sperm they receive alive during their reproductive lives. Of course, one of the greatest mysteries associated with honey bee mating biology is the ability of the queen to do this. Think about it: workers produced by a queen that is two years old are being sired by fathers who have been dead for two years! This is incredible and it is the result of the spermatheca's design. The queen's spermatheca is fitted with a two-armed gland (spermathecal gland) and a tracheal net. The former nourishes the living sperm while the latter provides the oxygen needed by the sperm to stay alive. The quality of sperm in the spermatheca tends to decrease as the queen ages.

Does sperm from multiple drones mix evenly in the spermatheca? In the oviducts, prior to movement of the sperm into the spermatheca, the sperm is not mixed evenly. In the spermatheca, on the other hand, the sperm does mix gradually, though not necessarily evenly. For example, a queen that mates with 8 drones would be expected to produce an equal amount of workers sired by each drone (i.e. 12.5% of the workers would be from drone A, another 12.5% from drone B and so on). However, this was not shown to be the case. It can take many months, or even a year or more, for the sperm in the spermatheca to mix fully. Thus, the answer to the question "does sperm mix evenly in the spermatheca" is "yes, eventually, though it takes some time." Given enough time, the sperm will mix nearly evenly in the spermatheca but that still does not mean that the same proportion of offspring will be produced by each father drone. After all, different amounts of sperm from each drone ultimately make it to the spermatheca.

The quality of a queen relies, in part, on the weather at the time she needs to be mated and the quality of the drones with which she mates. For example, if, because of poor weather, she fails to mate with enough drones, she can have a reduced sperm load and be more likely to exhaust her sperm supply quickly – thus becoming a drone layer. Likewise, the queen can mate with poor quality drones that contribute less than optimum amounts of or unhealthy sperm. Per Koeniger et al. (2014): "Drones with good, viable sperm are important for the production of high quality queens."

Drone sperm quality can be affected by a number of factors. The number of sperm produced per drone varies from 3-10 million sperm/drone. This number can vary greatly based on a number of external conditions. For example, drones developing in cold or hot brood nests have less viable sperm. Drones being parasitized by *Varroa* can have reduced sperm loads. Some have hypothesized that pesticides also may impact sperm loads in drones. These findings emphasize the need to invest in drone quality as much as one invests in queen quality if quality, mated queens are to be produced.

Conclusion

If the queen was successful on her mating flight(s), she will begin to lay her first eggs 12-15 days after emerging from her queen cell. For a while, the vast number of adult bees in the nest will be the new queen's brothers (drones) and sisters (workers) from the previous queen mother. Over time, however, the new queen's offspring will replace those of the previous queen and the colony becomes a direct reflection of the genetic makeup of the queen and the drones with which she mated. Fortunately, the majority of queens mate successfully and, thanks to their drone partners, continue on to lead productive colonies of their own.

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