

The Biological Basis for Breeding the Honey Bee

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There are three “pillars” to successful beekeeping: proper hive management, good locations that provide abundant nectar and pollen, and good quality queens. High quality queens are a product of both good queen rearing techniques and excellent genetic stock. This primer provides a framework for breeding honey bees, or more specifically, the deliberate selection and propagation of bees with superior combinations of genetic traits.

Breeding the honey bee has been a goal of both beekeepers and honey bee researchers for more than 150 years, but it is a subject that has not advanced as rapidly as the breeding of other livestock has. There are several reasons for this. First, brood viability declines quickly as inbreeding increases as a consequence of mating queens with drones that share some ancestors in common. Second, our rudimentary understanding of the genetics of honey bees, and more importantly of the inheritance of particular characteristics of bees, has severely limited progress towards fixing valuable characteristics in specific lines of bees. Third, multiple mating by queens with drones from many different colonies has limited our ability to control natural mating. Fourth, most traits of economic interest to beekeepers appear to be under the control of many genes, which increases the difficulty of successfully selecting for several traits simultaneously. Fifth, most scientific research on bee breeding has produced interesting information about specific characteristics (e.g., hoarding behaviour, alfalfa pollination, hygienic behaviour) but has not been designed to produce improved bee stocks; in contrast, commercial breeding operations lack the appropriate controls to allow their progress to be assessed. Finally, most books on the subject of bee breeding fail to go beyond the topic of queen rearing, are too general, neglect to explain the mechanics of how to conduct a breeding program, have unclear explanations of breeding practices, or are incomplete.

Several biological traits of honey bees create some interesting challenges and opportunities to individuals interested in improving them genetically. These include:

- Mating at drone congregation areas
- Multiple mating with on average 10-12 drones
- The ability to be instrumentally inseminated
- Polygenic inheritance of most of the many traits of interest to beekeepers
- Haplodiploid sex determination (males derived from unfertilized eggs)

Successful bee breeding requires working with these biological factors. In some cases, these biological characteristics can be used to advantage to enhance the breeding of honey bees. These four factors will be discussed below, following which several breeding schemes will be discussed.

Mating at Drone Congregation Areas (DCAs)

Honey bees are believed to mate at DCAs. These are localized aggregations of drones that form in the air in the same place year after year. Every afternoon under suitable weather conditions, drones move towards the hive entrance, then leave the hive. One school of thought is that they fly towards low points on the horizon and stop when they experience a particular set of optical conditions. Another is that they follow landforms such as roads, forest edges, valleys, and watercourses, and "DCAs" are the branch points along these aerial pathways where drones mill about. In either case, mature drones regularly fly 4 km (2.5 miles) from their nest, and are able to fly over 8 km (5 miles). This means that the drones a queen mates with can come from colonies over an area of 200 km² (80 square miles)! Moreover, because of this extreme level of mixing by drones, two queens that take mating flights from the same apiary are unlikely to mate with drones from the same colony.

Control over honey bee mating is impossible in many settings because of their mating system. One solution is to place mating nuclei and drone colonies in an isolated setting where no other honey bees are found. In Ontario, Sweden, and Denmark, placing the hives on islands has provided the isolation needed to control mating. In Egypt, desert oases have been used successfully. In Canada beekeepers can establish mating apiaries in northern areas away from commercial apiaries where cold winters have eliminated unwanted feral bee colonies. In general, control can be achieved in any setting where the mating colonies are 10 km (6 miles) from the nearest managed hives or wild colonies of bees.

Failure to control which males the queens mate with will severely limit progress in bee breeding. In that case selection will be exerted on queens only, which represent only half of total genetics of the worker bees. While some traits will be altered somewhat through selection only on breeder queens, one should not expect dramatic success in breeding when the drones available to mate with the queens are likely originating from 200-1000 different unselected colonies of bees.

Multiple Mating by Queens

After emerging and maturing a few days in the colony, a queen usually takes one or more short orientation flights. Then she leaves on a mating flight, during which she flies out, finds drones (probably in a DCA), and mates with several in succession. With modern DNA fingerprinting techniques, we know that queens of *Apis mellifera* mate on average with 10-12 drones during 1-3 mating flights.

During mating the seminal fluids of each male gets forced into the queen's oviduct which swells up to accommodate the large volume of material. A portion of it (about 10%) then migrates up the sperm duct into the round spermatheca, a sperm storage and nutritive organ that keeps the sperm alive for the life of the queen. During this process the sperm from different drones gets mixed to the extent that on a normal

day of egg laying, sperm from every male that mated with the queen fertilize some of the eggs.

This has important ramifications for bee breeding. A drone that mates with a queen contributes half the genes to the female offspring that he sires and affect their behavior. However, because of multiple mating, the performance of a honey bee colony represents the collective efforts of ten different sets ("patrilines") of worker bees, each of which has a different father, as well as the behavioral interactions between the bees of these different patrilines. When evaluating a colony headed by an open-mated queen, it is impossible to know how each patriline would have behaved on its own. Moreover, when rearing daughter queens from a colony with an open mated queen, the real father of each individual larva being grafted into a queen cell is unknown—it could be any one of the 10-12 drones the queen mated with! Imagine trying to breed horses and never knowing which of several stallions in the paddock mated with your prize-winning filly. It is unlikely the offspring of such crosses would ever win the Kentucky Derby!

Instrumental Insemination

Fortunately, our ability to instrumentally inseminate queen bees solves the majority of the constraints outlined so far. First, we can use several drones from a single colony to inseminate the queen. (A word of caution: because drone honey bees readily drift from hive to hive, it is necessary to confine them to the hive with queen excluders or hatch them in an incubator, then mark them with paint so there can be no question of their colony of origin.) Although the semen from drones from the same colony (i.e., brothers) is not genetically identical, they represent the genes of their mother only (because they develop from unfertilized eggs). Consequently, the offspring of queens inseminated by a set of brothers are much less variable than are the workers from open-mated queens.

Secondly, for some breeding purposes queens can be inseminated with the semen from a single drone. In this case the worker offspring will all be very highly related to each other, and one has complete control over the father of both the worker bees and new daughter queens reared from that queen's eggs. Colonies headed by single-drone-inseminated queens usually develop normally but never reach large size, and the queens tend to run out of sperm after a relatively short period of time. However, if the workers from such queens can be evaluated for specific traits without the colonies becoming large, single drone-insemination is the best method to identify traits of interest that can be incorporated into other stocks of bees.

Polygenic Inheritance of Many Different Traits

Many different traits affect the behavior of bees and the performance of colonies. Some are obvious, such as high honey production, excellent winter survival, and gentle behavior. None of these are simple traits, however, but rather are influenced by several genes. For example, honey production is affected by queen egg-laying rate, numbers of bees in the colony at the start of the honey flow, the ability to work different types of flowers, tongue length that influences the ability of the bee to reach the nectar, the efficiency of recruitment to floral resources through dances, the rapidity with which hive bees relieve foragers of their nectar loads, etc. Queen

producers are concerned with the ability of bees to accept larvae into queen cups and rear them into queens. Package bee producers want large bee populations early in the spring. Everyone now is interested in resistance to diseases such as AFB (especially now that it is becoming resistant to antibiotics) and parasitic mites. The point is that there are a very large number of traits of interest to beekeepers.

From the bee breeder's standpoint, this creates a large problem. As the number of traits of interest to the breeder increases, the possible number of genetic combinations increases exponentially. To be effective, breeders need to limit the number of traits upon which they base their selection of breeder queens. It is not possible to effectively select for more than four or five different traits simultaneously.

One partial solution to this problem is to select intensively for a single trait, such as tracheal mite resistance (which also is influenced by a number of genes). With proper selection and good control on mating, it should take only a few generations to enhance mite resistance to the point that tracheal mites no longer pose a significant problem to the beekeeper. Once this trait is fixed in a breeding population (e.g., all bees express high levels of resistance), it can be bred into other stocks of bees through deliberate crossing and rigorous selection for several more generations.

Haplodiploidy and its Consequences

Sex Determination

This is the most difficult of these concepts to explain, but it has far reaching implications. Male bees are haploid, meaning that they have only one set of chromosomes as a result of developing from unfertilized eggs. A male consequently gets all his genes from his mother. Females (queens and workers) are diploid, having two sets of chromosomes, one set contained in the egg derived from the queen and one from the male's sperm that fertilizes the egg. Thus bees are referred to as haplodiploid.

The real situation is even a bit more complex. Bees have a sex gene, often referred to as "X" in books. That gene exists in many different forms, or alleles (X^A , X^B , X^C , etc.). Most populations of bees have about 10-12 sex alleles. To be female, a bee must be heterozygous at the sex allele, meaning that the sex alleles obtained from the queen and drone must be different (e.g., $X^A X^B$, $X^A X^C$). To be male, a bee must have only one type of allele at the sex gene, but that can come about in two ways. The normal way in a bee colony is by the queen closing the sperm duct as an egg is laid, resulting in an unfertilized egg (i.e., X^A or X^B). It is also possible for a fertilized egg to be genetically "male" if the egg is homozygous at the sex gene (e.g., $X^A X^A$). This egg could develop into a "diploid drone" if reared artificially, but the workers within the colony cannibalize the larva shortly after hatching.

Inbreeding

Inbreeding is the crossing of a queen with drones that share some ancestors in common. Virtually all classical breeding programs of livestock and crops rely on inbreeding to some extent. This happens automatically in bee breeding as a result of selectively propagating the offspring of a relatively few individuals. By selecting only a few breeder queens in each generation, the progeny will begin to share more and more genes in common because they are descended from these few ancestors

(breeder queens). Some crops (e.g., corn, wheat) are inbred to a high degree by breeders. Among the thousands of plants they may plant after making such a cross they can select the few with the desired characteristics. After a few generations of selection most genetic variation will be eliminated and the offspring will be homozygous for most traits. That is, the breeders will have fixed the traits of interest in the offspring so subsequent crosses will breed true to the traits selected in the parents.

The negative aspect of inbreeding is that the consequent increase in homozygosity leads to inbreeding depression (the opposite of hybrid vigor) in most species. This is particularly true for honey bees due to their sex determination system. As one begins to make specific crosses (non-random mating) and propagate the offspring from them, inbreeding occurs. While inbreeding helps in the selection of specific traits in the bees, it also leads to higher percentages of fertilized eggs being homozygous at the sex gene and consequently being inviable diploid drones. As inbreeding continues, the vigor of the colony declines. Part of this is due to the increasingly larger percentage of diploid drone eggs being laid in worker cells. Although the queen lays eggs in these cells after the diploid drone larvae have been eliminated, the loss of larvae has a negative influence on the growth of the colony and causes a "shot brood pattern" with open cells interspersed among sealed brood cells.

Beekeepers can slow the development of homozygosity at the sex gene in several ways. When choosing breeder queens it is generally advisable to select colonies that exhibit very few empty cells among the brood. By doing this they maintain the maximum number of sex alleles in their breeding population. It is not advisable to use the same queen as a breeder in succeeding generations, as this is likely to result in matings between brothers and sisters. Similarly, the use of the same drone-producing colonies in two or more successive years should be avoided, because it could result in matings between uncles and their nieces. Exchanging a few breeder queens with other beekeepers every few years will generally introduce additional sex alleles into the breeding population and will translate into more consistent brood patterns.

Dr. Rob Page, University of California-Davis, developed the concept of the closed breeding population which is the best means of selecting and improving a population of bees while minimizing the effects of inbreeding (i.e., the loss of sex alleles). Under this scheme, a population of bees would be selected to initiate a breeding program. Depending on the goal of selection these could be either randomly selected colonies or colonies chosen for specific traits they exhibit. The initial population should consist of at least 25 colonies; more than that will increase the amount of time before inbreeding and diploid drone production become a serious issue. Every year a group of five or more daughter queens would be produced from each colony within the breeding population. These would be either instrumentally inseminated with homogenized semen taken from drones of all colonies in the population or allowed to naturally mate in an isolated setting with drones of all colonies. After evaluation for the traits of interest and brood viability, the queen in each hive would be replaced by the best of her daughters (e.g., queen supersedure). Page developed several modifications of these scheme. Closed population breeding has been used successfully to breed the Alberta Bee in Canada, to improve honey

production in western Australia, and in commercial development of the New World Carniolan Bee.

Sperm Cells of a Drone are Clones of the Drone Itself

As explained earlier, drones develop from unfertilized eggs and have only one set of chromosomes. Their sperm must also have a single set of chromosomes because production of diploid (female) offspring requires fertilization of the haploid egg by a haploid sperm. Consequently, because both the drone and his sperm are haploid, each sperm cell is genetically identical to the drone himself. This characteristic of bees can be used to advantage by bee breeders because the worker offspring of single-drone-inseminated queens share a higher proportion of genes in common (3/4) than is found between sibs of most other organisms. It is thus highly likely that the desirable traits identified in the workers of a colony will be present in new queens reared from the same single-drone-inseminated queen.

Conclusions

If these underlying principles of bee breeding are understood, then the specifics of various mating systems begin to make much more sense. A few final pointers may help.

1. Devise simple, quick ways to evaluate the traits you wish to breed for. It is essential that your evaluation gives unequivocal results, yet does not take up too much of your time and effort.
2. Progress in breeding will be slow and will never achieve the desired results if there is no selection of drones (i.e., control over mating). Isolated mating apiaries and/or instrumental insemination must be used to eliminate mating with unwanted males.
3. Be aware of the degree of brood inviability in your bees. If the sealed brood patten begins to decline (below 90%), introduce some new stock in your breeding population to increase the number of sex alleles.

With consistent effort you will shape the characteristics of your bees such that they will perform better for you under your conditions.

There is presently only one book that adequately explains the basics of bee breeding as well as queen rearing techniques. It is:

Harry H. Laidlaw Jr. and Robert E. Page Jr. 1997. Queen Rearing and Bee Breeding. Wicwas Press, Cheshire, Connecticut.