An Introduction to Honey Bee Breeding Program Design

Honey bee breeding is a long-term, labor-intensive process that is becoming more popular and accessible.

Breeding Program Basics

Evaluating colony performance and making informed selections of breeding stocks requires meticulous planning, rigorous record keeping, and regular inspections. Because honey bee queens naturally mate in flight with multiple males of unknown lineage, making controlled crosses requires specialized training and equipment.

Image 1. Honey bee breeders select the highest performing colonies to use for queen and drone production. Image: Kate Anton, Penn State

The foundation of a successful breeding program is laid with careful evaluation and trait selection. Selecting for desirable traits such as honey production, temperament, Varroa mite resistance, and other attributes require the beekeeper to keep detailed accounts of colony condition and behavior throughout the active season and track changes over time. Additionally, the environment and function of the colonies should be considered. For example, honey bees used in commercial pollination face different stressors than those that live in stationary locations and preferences for specific traits vary among individual beekeepers. In this way, breeding program design and objectives are based on the needs of the breeder and their client base.

Getting Started

To implement a breeding program, some beekeepers begin by purchasing queens which are proven to have desirable traits as a starting point while others work with existing stock. Dr. Marla Spivak and Gary Reuter (2021) suggest screening the highest performing colonies and rearing queens from colonies that have never exhibited signs of chalkbrood as a starting point for selecting for hygienic behavior. Successful selection requires the beekeeper to cull queens or even entire colonies that exhibit unacceptable behaviors (such as high disease presence or extreme defensiveness) in order to remove the offspring from those colonies from the reproductive pool.

Figure 1 demonstrates the structure of a breeding program used in a research study: *Genetic parameters of honey bee colonies traits in a Canadian selection program*. This study began by using 26 mother colonies from Canadian and Danish breeding stock which were evaluated to select the queen and drone producers. From these colonies, the first generation of 100 colonies for the experiment were generated (Maucourt et.al., 2020). This experiment demonstrated positive correlations between many desirable traits and also shows the importance of evaluation and selection for improving colony performance.

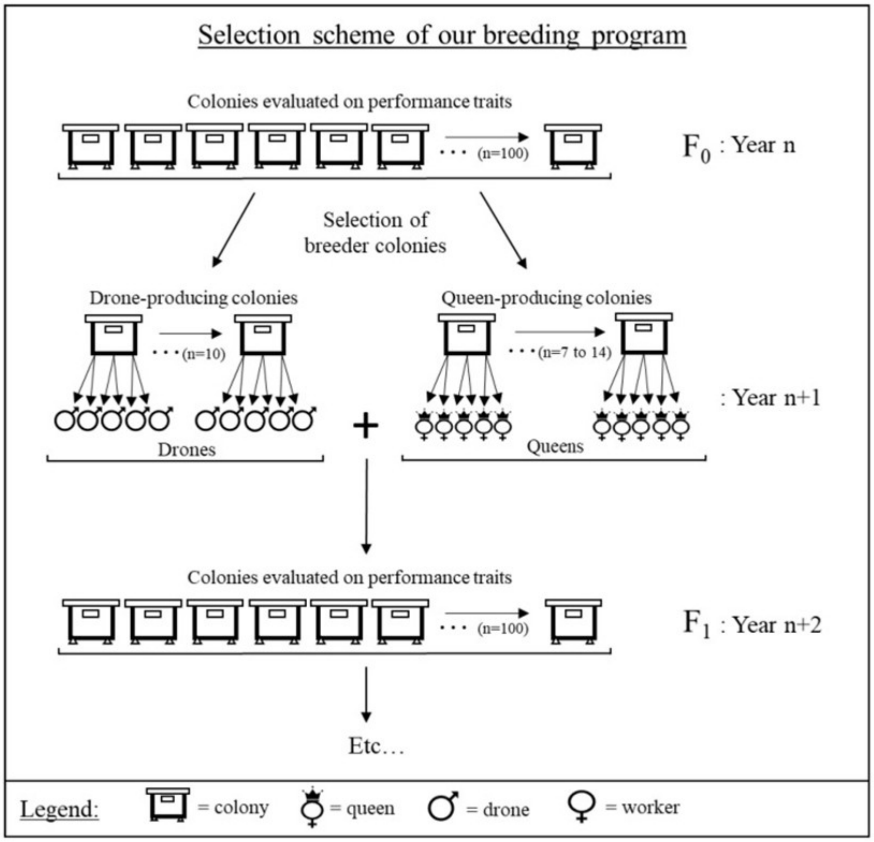

Figure 1. Selection scheme of a breeding program. Colonies of the F0 generation were evaluated on their performance traits (year n). About twenty breeder colonies were selected from this F0 generation (year n+1): between 7 to 14 colonies were selected to produce drones, and 10 were selected to produce queens. The queens and drones produced were mated together to constitute the F1 generation, which was evaluated on performance traits in year n+2 (Maucourt et.al., 2020).

Types of Breeding Systems

Breeding programs are described as closed or open systems. Closed breeding systems typically use instrumental insemination (artificial insemination) to control the mating between selected queens and drones. This technique requires advanced training and specialized equipment and is rapidly gaining popularity. Closed breeding systems allow breeders to rapidly produce colonies with desirable genetic traits by controlling mating between queens and drones and rearing new queens and drones from these crosses.

Image 2. In closed breeding systems, drones are reared and collected from selected colonies for use in instrumental insemination. This apiary is exclusively for drone-producing colonies to ensure that each drone is sourced from desirable stock. Image credit Kate Anton, Penn State

Open breeding systems can be used in conjunction with closed systems or be implemented independently. Open systems rely on queens mating naturally with drones in the vicinity, making it more accessible to beekeepers who don't have access to the resources required for a closed system. Rearing queens from the top-performing colonies and requeening poor-performing colonies with desirable stock can improve the overall quality in the apiary. Additionally, introducing drone-producing colonies across a region helps improve traits by flooding the mating pool with desirable drones. The degree of precision and success of an open mating system is influenced by the local environment and scale. Specifically, the ratio of desirable drones in the local population who can mate with queens will determine the degree of success. Local beekeepers and organizations can work together to introduce desirable genetics in a region by supporting a local breeder or distributing queens through a cell program.

Genetic Diversity

Breeding programs require sufficiently large numbers of colonies to provide genetic diversity. Inbreeding causes low genetic diversity and reduces overall colony performance. For example, colonies with a high degree of genetic diversity have been shown to have higher disease resistance than colonies with low diversity. Additionally, inbreeding can result in the queen laying diploid eggs (which normally would develop as workers) that have two copies of complementary sex determination genes. This causes fertilized eggs to develop into drones rather than workers. Diploid drone larvae are removed by the workers soon after hatching, resulting in a "shot brood" pattern and a reduced population of new workers to support the colony. Thus, honey bee breeding operations need to ensure that they maintain and breed from a genetically diverse population of honey bees.

Honey bees are not native to North America. Of the 28 subspecies found in Europe, Africa, and Asia, only a small number of several subspecies was introduced to the U.S. before the 1922 law restricting importation. Therefore, the genetic diversity of US honey bee populations is limited.

Washington State University runs a program dedicated to increasing genetic diversity in the United States. Honey bee germplasm (bee semen) from several Old World subspecies has been collected and imported, under APHIS permit, and incorporated into US commercial bee breeding programs. Germplasm from these collections has also been cryogenically preserved for future use and conservation.

Image 3. Image of an instrumentally inseminated Carniolan queen bee. Image: Kate Anton, Penn State

Common Traits Targeted by Breeding Programs

Desirable colony-level traits in honey bees represent a collection of behaviors expressed by individual bees in the colony. Many beekeepers are interested in selecting for "locally adapted stocks" which include a number of different traits including colonies that are docile, build up rapidly in the spring, have high honey production, and high winter survival. These colony-level traits are often associated with resistance to disease and parasites. For example, Varroa mite resistant colonies can produce longer-lived winter bees, leading to improved winter survival, faster spring build up, and higher honey production in the summer.

Many breeding programs focus on evaluating and selecting genetic stocks that display Varroa mite resistance, which can reduce the need to apply chemical treatments and can increase productivity and survival of honey bee colonies. Evaluation of these traits can be conducted throughout the season and completed in a relatively short time frame (1 day to 1 week). In addition to monitoring overall Varroa levels and comparing between colonies, specific types of Varroa mite resistance can be categorized and or tested in one of three ways; hygienic behavior, Varroa sensitive hygiene (VSH), and grooming.

Hygienic behavior describes the ability of individual nurse bees to detect a diseased or damaged larvae or pupae within a capped cell and remove them. Minnesota Hygienic bees are an example of a successful breeding effort founded by Dr. Marla Spivak who continued research into hygienic behavior pioneered by Dr. W. Rothenbuhler in the 1960's. Screening for hygienic behavior is accomplished through freeze kill tests using liquid nitrogen or installing previously frozen sections of capped brood into colonies and documenting the quantity of dead brood removed after 24 hours. A colony is considered to be hygienic if it scores at least 95% on two consecutive tests. More information on testing hygienic behavior can be found in the SARE Fact Sheet, Testing Honey Bee Colonies for Hygienic Behavior.

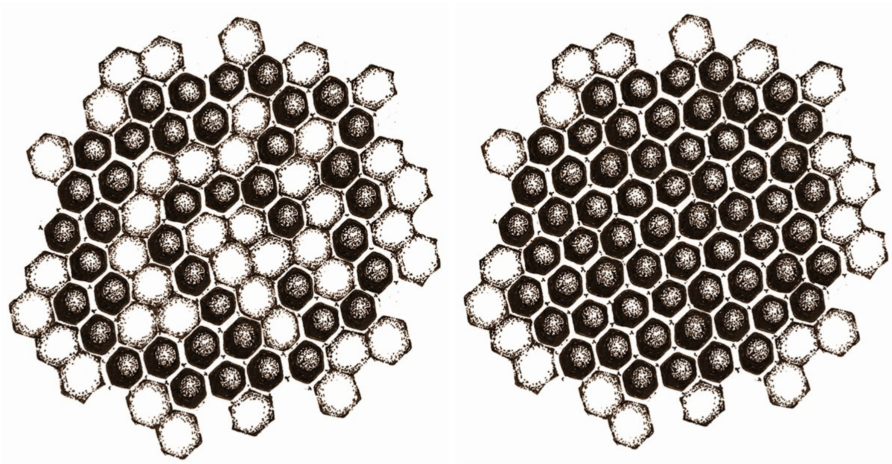

Image 4. Comparison of hygienic behavior using a freeze kill test. The image on the left shows a non-hygienic colony with a removal rate of 80%. The image on the right shows a hygienic colony with a 100% removal. Illustrations by Michael Hill, Penn State

The term Varroa sensitive hygiene (VSH) was coined by Dr. Jeffrey Harris at the USDA Baton Rouge bee lab to better describe this hygienic trait. Originally, researchers thought that the bees were behaving in a way that decreased the mites' ability to reproduce and called it suppressed mite reproduction (SMR). Later, it was discovered that VSH is a form of hygienic behavior where the workers remove pupae infested with reproductive mites and their offspring. Interestingly, non-reproductive (infertile) mites, which occur naturally in the population, are not removed and remain in the colony - and are therefore detectable during evaluation in colonies expressing high levels of VSH.

VSH expression is scored by examining frames of capped brood with a microscope or magnifying lens and counting the number and reproductive status of Varroa mites. According to the protocol developed by Dr. John Harbo, a pioneer in VSH research, a colony is considered 100% VSH if only non-reproductive mites are found during evaluation. Information about measuring VSH can be found on this website.

Additionally, a behavior known as recapping can be a source of confusion in regard to VSH and hygienic behavior. Recapping occurs when workers uncap and recap healthy brood cells. This behavior is frequently but not necessarily observed in VSH bees. Recapping was once hypothesized to be linked to VSH and involved in changes to mite reproduction. However, researchers have not been able to demonstrate a link between recapping and a reduction in mite fertility. Because not all VSH bees perform this behavior, recapping is not considered to be genetically linked to VSH.

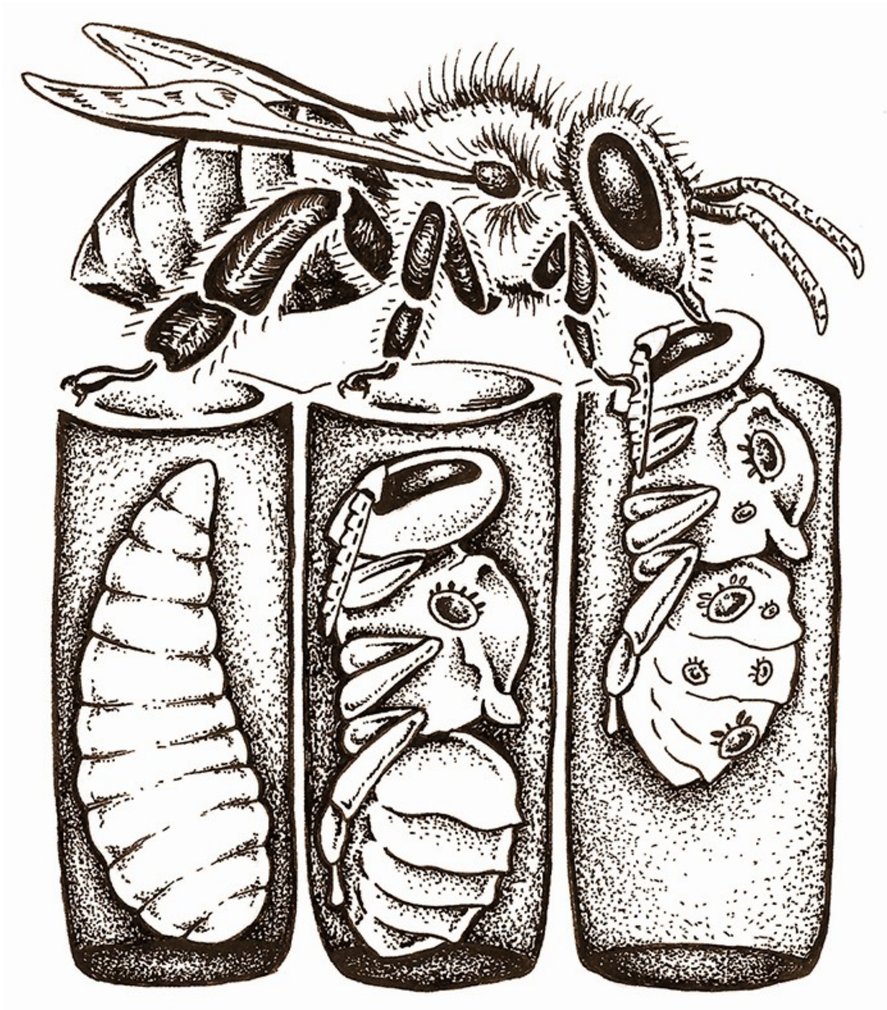

Image 5. VSH bees detect and remove pupae with reproductive Varroa mites and their offspring. Illustration by Michael Hill, Penn State

Grooming behavior is characterized by the bees' ability to remove mites from themselves (autogrooming) and their nestmates (allogrooming) with their legs and mandibles. This behavior is well documented in Apis cerana (Asian honey bee) and has been observed in some stocks of European honey bees (Apis mellifera).

A specialized form of this behavior, known as mite *biting* or *mauling*, occurs when workers use their mandibles to chew and therefore damage the legs of the Varroa mites, rendering them immobile. Testing for this behavior is performed by sampling mites that fall onto a sticky board and measuring the proportion of mites with missing or damaged legs or other body parts using a microscope. The breeding program at the Purdue University Honey Bee Lab, currently headed by Dr. Brock Harpur, has been selecting for this behavior since 2007. More information on mite biting can be found on their Honey Bee Extension website.

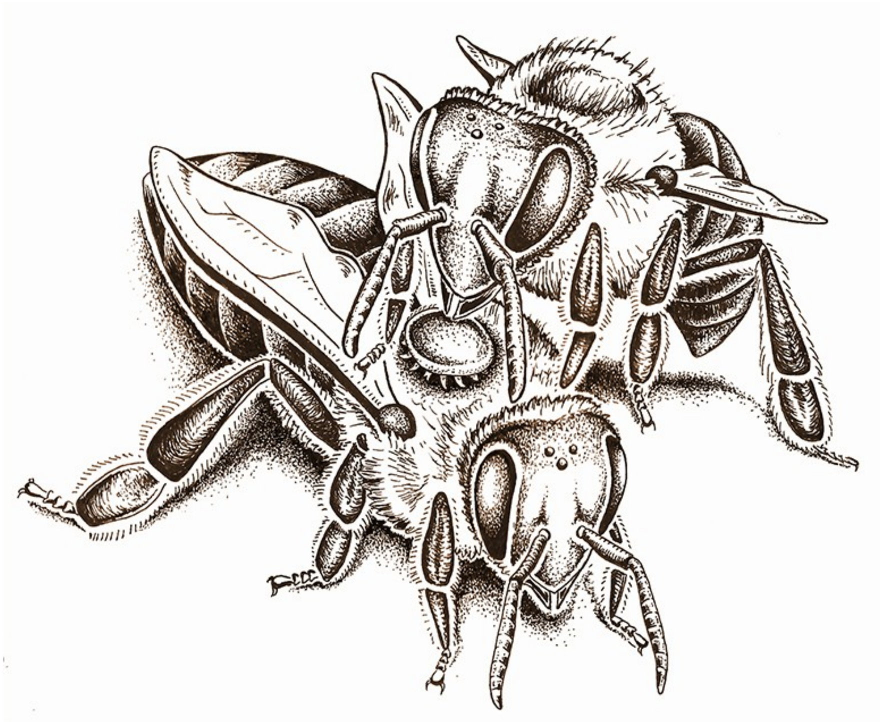

Image 6. Allogrooming behavior occurs when one bee grooms another. Illustration by Michael Hill, Penn State

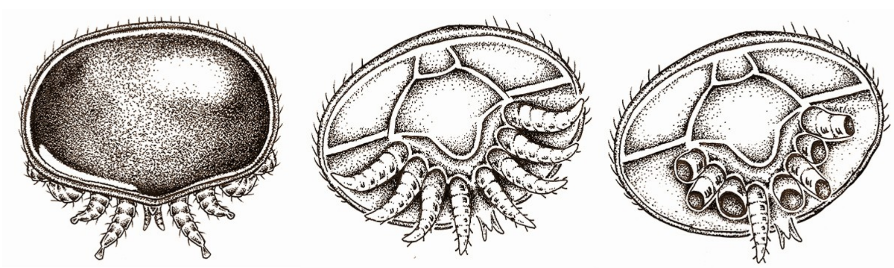

Image 7. Varroa mite from left to right; dorsal view of intact mite, ventral view of intact mite, and ventral view of mite with missing and damaged legs as a result of chewing. Illustrations by Michael Hill, Penn State

It is important to note that screening for behavioral traits alone does not guarantee resistance to Varroa mites or disease. In the case of hygienic traits, the timing of brood removal is critically linked to effectiveness of disease control. Some colonies who perform very high on freeze kill tests may not remove infected brood early enough to prevent diseases like chalkbrood and American foulbrood. Testing for behavioral traits should only be used as one part of the screening process for choosing breeding stock. Breeding programs should integrate regular inspections, performance evaluations, and Varroa mite population sampling along with testing for behavioral traits to select the highest performing stock.

Linked Traits

There are many linked colony-level traits that beekeepers consider beneficial. For example, researchers in Turkey (Ahmet and Hakan, 2013) evaluated 90 colonies of honey bees through an entire season and discovered that colonies with higher hygienic behavior produced more wax and honey, and had a higher adult bee population. Moreover, bees from colonies that exhibit high levels of defensive behavior have been found to have lower mite populations and higher resilience against pesticide exposure (Rittschof et. al., 2015). Many research groups across the world are actively studying how genes, chemical signals, and environmental conditions interact to influence the health and behavior of honey bees and other animals.

Research conducted by Dr. Tom Seeley's lab at Cornell University explored colony 'personalities' and noted that colonies with higher foraging activity tended to have a higher defensive response and removed dead bees more rapidly. They also observed that runniness (workers who tend to run across comb) was linked to defensive response and poor repair of damaged comb. Despite sharing a high degree of defensiveness in common, colonies with the tendency to exhibit runniness did not demonstrate high foraging levels (Wray et. al., 2011). Clearly, honey bee breeding is not a simple process, defensive behavior can be linked to both desirable traits (high foraging, low mite populations) or undesirable traits (runniness, poor comb repair). Because some traits are often observed together, breeders must have an evaluation process that weeds out unacceptable behaviors while selecting for beneficial ones.

Most beekeepers are able to judge colony temperament through experience and comparison with neighboring colonies. Researchers study honey bee defensive response by using specially designed tests, one test uses a chemical that is the main component of alarm pheromone. When the chemical is applied to the hive entrance, responsive bees will gather and can be counted. Another test involves waving a patch of fabric in front of the entrance after disturbing a colony and counting the number of times the patch was stung.

Image 8. An entrance of a colony before (left) and after (right) application of chemical alarm pheromone. Image: Clare Rittschof

Image 9. Patches used in a behavioral test. For this test, a small piece of fabric is waved in front of a colony that was disturbed. The number of stings can be counted to evaluate defensive response. The patches on the left and middle have few stings and the patch on the right has many indicating that the colony on the right had a higher level of defensive response. Image: Clare Rittschof and Robyn Underwood

Evaluation and Interpretation

To establish a breeding program, the beekeeper must identify desirable traits and methodologies for testing. Selection may be based on several traits or focused on a single trait such as hygienic behavior. Even while selecting for a single trait, it is important to have criteria excluding colonies which exhibit unacceptable characteristics, such as extreme defensive behavior or poor disease tolerance. Successful programs track colony performance throughout the seasons and over the course of many years.

Monitoring and selecting for colony-level traits (temperament, spring build up, honey production, winter survival) is time consuming and rigorous. The local environment and time of year will be the strongest influences, so performance should be judged against other colonies in the same apiary and compared at similar time points. For example, colonies in an area with poor floral resources will have low honey production regardless of a colony's genetic background. Furthermore, colonies near other beekeeping operations may have increased mite populations because of the proximity to poorly managed stock. Performance evaluation can range from testing a single stock to comparisons of multiple stocks, it is important to note that evaluation becomes increasingly complicated as the number of stocks and locations increase.

Notably, the degree of Varroa mite resistant behavior can vary throughout the season and is impacted by environmental conditions. It is important to evaluate colonies simultaneously and repeatedly especially when comparing performance across multiple locations. For example, a freeze kill test for hygienic behavior should be repeated to confirm a colony exhibits the behavior consistently. It is not uncommon to find significant differences in the expression of hygienic behavior at different times of year or when colonies are stressed by the landscape or the beekeeper.

When testing for mite resistance along with other traits, it is important to make sure the nurse bees in the colony are the daughters of the queen. Since hygienic and grooming behavior is performed by nurse bees, the queen must have been laying in the colony for at least six weeks before testing can provide accurate results.

Table 1 is an example of a field data sheet that a beekeeper may use to evaluate the performance of their colonies. After collecting colony data, the scores can be entered into a spreadsheet to identify top performing colonies to use as breeding stock.

Table 1. A field data sheet used during colony inspection to evaluate performance (K.Anton, R. Underwood)

- **Name:**
- **Date:**

- **Queen:**
- **Location:**

Trait/Score	**1 (cull)**	**2**	**3**	**4**	**5**
Temperament	Aggressive	Agitated	Medium	Docile	Extremely docile
Honey Production/Stores	No stored resources	Low	Medium	Great	Excellent
Brood Production	No brood	1-2 frames with brood	2-4 frames of brood	4-6 frames of brood	More than 6 frames of brood
Disease	More than 10 cells with disease	3-10 cells with disease	Less than 3 cells with disease		No signs of disease
Mite Population	5+ mites/100 bees	3-4 mites/100 bees	2 mites/100 bees	1 mite/100 bees	0 mites/100 bees
VSH Score	0	1	2	3	4

Interpreting the data requires the beekeeper to consider the context of each evaluation. As we discussed earlier, the local environment is a powerful influence and must be a factor when considering colony performance. A preset list of desired traits and a system for evaluating performance will identify colonies that don't measure up and those which rise above the rest.

Challenges

Breeding efforts in honey bees have the promise and potential to improve beekeeping outcomes when they are successful. There are many examples of successful breeding programs; Minnesota Hygienic bees, the USDA's Pol-line, and the Purdue mite biters are among the most well known examples. There also are numerous challenges to establishing and maintaining a successful breeding program.

Honey bee breeders must invest in education, training, equipment, colonies, and labor to achieve satisfactory results and it often takes many years before a return on investment can be realized. Historically, breeding programs have been established by universities and government agencies that could offer the support needed to make advances in this field. The work of these research programs has and continues to advance understanding, knowledge, and techniques - making it possible for independent operations to expand into the field, though there are still significant obstacles. Most notable is the number of colonies required for a successful breeding operation, typically breeders have access to hundreds or even thousands of colonies to select from in order to refine traits. Smaller operations may need to expand or work cooperatively with other beekeepers to overcome this threshold. Beekeepers in colder climates face the additional challenge of a shorter breeding season and migratory beekeepers must contend with the demands of their pollination contracts. Certainly, there are many benefits to obtaining and improving stocks, but managing a breeding program is not a feasible option for most beekeepers.

Image 10. Breeding programs require many years of commitment, training, rigorous work, and large numbers of colonies. Image: Kate Anton, Penn State

Conclusion

Establishing a honey bee breeding program requires planning, organization, and dedication. While honey bees are undoubtedly an essential part of our agricultural system, simplified breeding programs like those for crops or cattle do not exist for honey bees. Honey bee breeders must integrate advanced management practices with a knowledge of genetics in order to design a successful program.

Image 11. An instrumentally inseminated queen with a retinue of workers. Image: Kate Anton, Penn State

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This material is based upon work supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, through the Northeast Sustainable Agriculture Research and Education program under subaward number LNE22-447.

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

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Penn State College of Agricultural Sciences research and extension programs are funded in part by Pennsylvania counties, the Commonwealth of Pennsylvania, and the U.S. Department of Agriculture.

Code: ART-7952