BREEDING AND GENETICS OF HONEY BEES

BY JOHN R. HARBO AND THOMAS E. RINDERER[1]

Domestic animals, such as chickens, cattle, sheep, pigs, and horses, have been selectively bred by human beings for thousands of years. Consequently, when modern breeding practices came into use, much selection had already been done; the modern animal breeder began with human-selected "breeds." The races of honey bees (such as Caucasians, Carniolans, and Italians) often are regarded as one would regard breeds of cattle or dogs. They should not be, for the honey bee races were not strongly controlled and bred by people and are much more variable than a breed of domestic animal.

The honey bee was not strongly selected by humans because basic bee reproduction was not understood until 1845. Without this understanding, very little could be done. In 1851, when this basic understanding was becoming widely accepted, Langstroth developed the movable frame hive. Suddenly beekeepers not only understood bee reproduction, they could also manipulate the hive and control the queen.

Controlling mating was the only obstacle remaining. Island isolation was one means, but it was of very limited value. Between 1860 and 1940, dozens of attempts were reported to induce queens and drones to mate in the confines of a jar, cage, tent, or greenhouse. Some claimed success, but the successes could not be verified or repeated. With the development of instrumental insemination as a practical technique in the 1940's, controlled bee breeding began.

Therefore, as people begin breeding bees, they enjoy the benefits of having a large and variable population with which to work. Breeders quickly discover that honey bees respond well to selection. In part, this is because human beings are just beginning to modify the bee through selection and controlled breeding.

[1] Research entomologist and research geneticist, respectively, Science and Education Administration, Bee Breeding and Bee Stock Research, Baton Rouge, La. 70808.

Genetics of the Honey Bee

The family relationships within a colony of bees are different from other agriculturally important animals as a consequence of mating habits, social structure, and drones developing from unfertilized eggs. The honey bee colony found in nature is a complex family group, best described as a *superfamily*. This superfamily (fig. 1) consists of: (1) one mother queen, (2) several father drones present as sperm in a sperm storage organ (spermatheca) of the queen, and (3) the worker and drone offspring of the mother and fathers.

Within a superfamily are usually 7 to 10 *subfamilies*, that is a group of workers fathered by the same drone. Since all the sperm produced by a drone are genetically identical, each subfamily is composed of sisters that are more closely related than full sisters of other animals. Thus, workers belonging to the same subfamily, often called *supersisters*, have three-quarters of their genes in common by descent. They receive identical gametes from their father and, on the average, half-identical gametes from their mother.

Workers belonging to different subfamilies have the same mother but different fathers. They are half sisters and have one-quarter of their genes in common by descent. On occasion, brother drones mate with the same queen. In such instances, their subfamilies are related to each other as full sisters rather than half sisters. Through natural mating, such full sisters probably are uncommon.

Despite the complicated family structure, the basic principles of genetics still apply to bees. The chromosomes contain hereditary units called *genes*. The specific place on a chromosome where particular genes are found is called a *locus*. On rare occasions, a gene entering an egg or sperm has changed somewhat and will have a different effect than the original gene. The process of change is called *mutation*, and all the forms of a gene that might occur at a locus are called *alleles*.

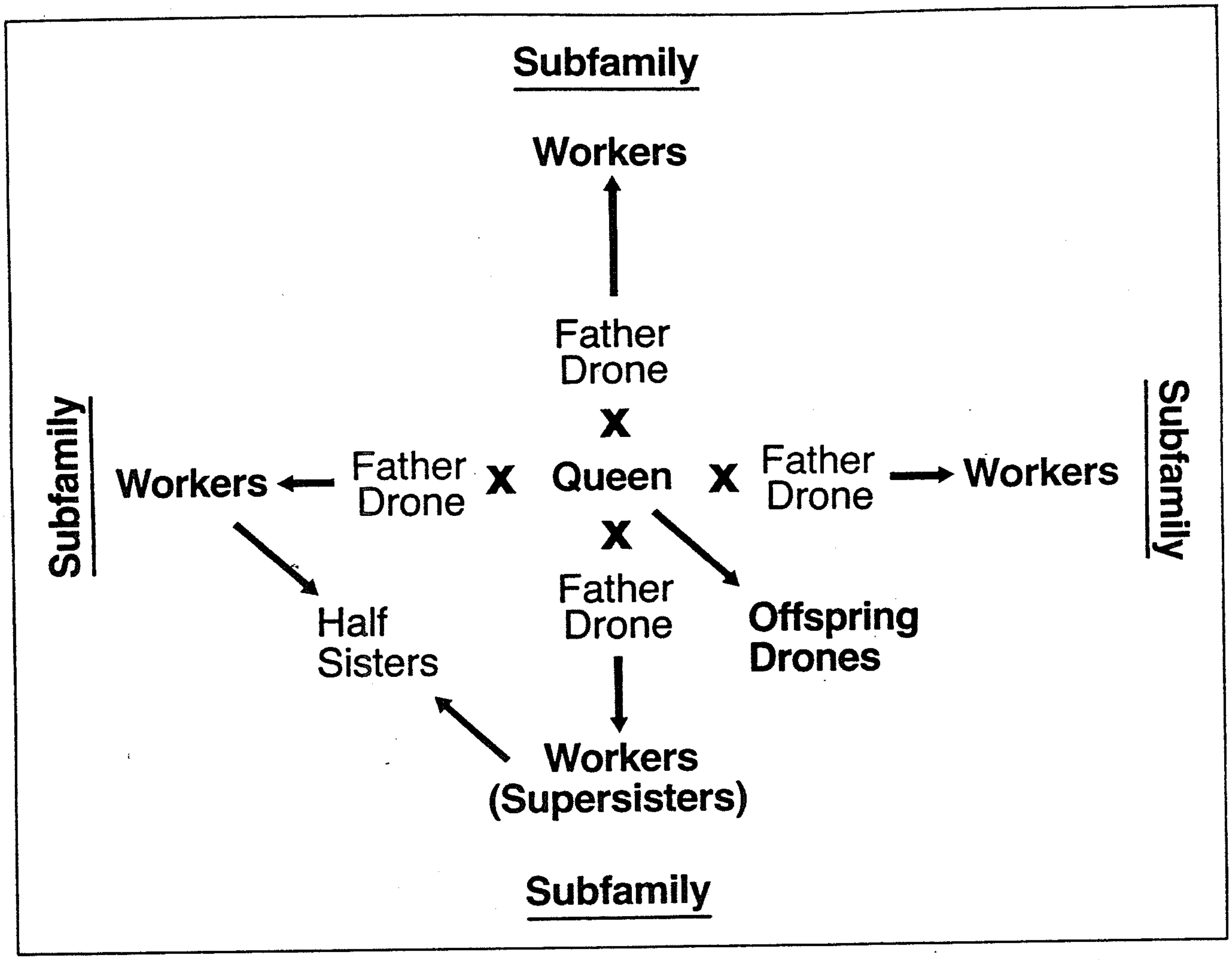

FIGURE 1.—A representation of a colony of bees as a genetic superfamily. The colony in the figure has four subfamilies, but there can be more or fewer. If two father drones are themselves brothers, the two subfamilies sired by them would be related as full sisters, rather than as half sisters.

Honey bee eggs hatch regardless of whether they are fertilized. The female bees—queens and workers—develop from fertilized eggs that contain 32 chromosomes. These 32 chromosomes consist of two sets of 16, one set from each parent. Hence, female bees are said to be *diploid* in origin. The males (drones) develop from unfertilized eggs which contain only one set of 16 chromosomes from their mother. Drones are thus *haploid* in origin. This reproduction by the development of unfertilized eggs is called parthenogenesis

Since queens and workers have paired chromosomes, they carry two alleles for each gene, one on each member of the pair. If both alleles are of the same type, the condition is homozygous; if they are different, the condition is *heterozygous*. In some heterozygous circumstances, one allele will mask the expression of the other and is said to be *dominant*. The allele which has its expression masked is said to be *recessive*. Drones can carry only one type of allele because they are haploid; thus, they are called *hemizygous*.

At one time parthenogenesis was considered to be the basis of sex determination in bees. The theory was that a chromosome dosage effect occurred such that two sets of chromosomes resulted in females and one set resulted in males. While this is a reasonable explanation, this theory is now known to be untrue.

Research workers investigating apparent low egg viability in inbred lines discovered that sex in bees is determined by the alleles at a single locus.

If an egg is a heterozygote at this locus, it will develop into a female. If it is homozygous or hemizygous, it will develop into a male.

The apparent nonviable eggs found in the inbred lines were diploid eggs homozygous at the sex locus. Worker bees selectively remove and destroy homozygous diploid larvae from the comb just after they hatch. Research efforts have been made to rear these diploid drones to maturity with the hope of producing diploid sperm and triploid queens and workers. However, artificial rearing is a difficult procedure, and the resulting diploid drones had reduced testes and produced very little sperm.

While sex determination is genetically complicated, other characteristics can be even more complicated. Different combinations of alleles at a locus result in different expressions of characteristics. Alleles at other loci also can affect a characteristic. All these different events result in complex genetic systems which produce a wide variety of character expression in bees. For example, worker bee response to isopental acetate (a component of alarm pheromone) was estimated to be influenced by at least seven to eight genes. This variety is some of the raw material necessary for the genetic improvement of bee stocks.

As with other animals, variety in bees is further increased by events that occur when a female (queen) produces an egg. During this time pairs of chromosomes in cells that are destined to become eggs exchange segments. Further in the process, the chromosome number of germinal eggs is halved. This process results in a haploid egg, with chromosomes having a new combination of alleles at the various loci.

Unfertilized, the egg will develop into a drone which will produce sperm. The processes of recombination of alleles and reduction of chromosome number do not occur in drones. All the sperm cells produced by a drone are genetically identical. They are identical to each other, and they are identical to the chromosomes in the unfertilized egg that developed into the drone.

Communicating Pedigrees

Like other animal and plant breeders, bee breeders need a simple format for communicating ancestry and breeding plans. A pedigree format usually is a standardized diagram, simply showing a line from the father and one from the mother to one or more offspring. Because of the haploid drones, bee pedigrees are different.

To properly discuss a pedigree, two terms must be defined. These are *gamete* and *segregation*. An animal gamete is an unfertilized egg or a sperm cell containing half the chromosomes needed to produce a worker or queen. Segregation is the random sorting of paired chromosomes to produce gametes. In most animals, segregation occurs in the ovaries and in the testes. In bees, segregation occurs only in the ovaries of queens.

Therefore, in honey bees, all new gametes originate with a queen. We say "new" gametes because drones propagate only existing gametes. The drones then have two reproductive functions: first, they convert and extend the queen's female gamete (the single unfertilized egg that develops into a drone) into about 10 million identical male gametes (sperms). Second, they serve as a vehicle to move the propagated gametes to the queen (the act of mating).

In bee reproduction, then, the female progeny receive one gamete from the queen that produced the egg and the other gamete from another queen (via drone conversion of the gamete to a sperm cell). Thus, a bee pedigree contains only females. So instead of using the traditional circle to represent females and the square to represent males, the bee pedigree in figure 2 has only circles (ovals), for only females (or queens) need to be recorded.

Stock Propagation and Maintenance

Controlled Mating

Some degree of controlled breeding has been practiced by queen producers for over 75 years. During that time, beekeepers had the capability of producing hundreds of queens from a selected colony rather than relying on natural supersedure or swarming. Thus, the female line was controlled.

Controlling the mating has been possible only by establishing isolated mating yards or through instrumental insemination. Isolated mating yards have two major shortcomings: (1) Absolute control of matings is difficult to achieve because a queen can mate with drones that are up to 5 miles away, and (2) one isolated mating yard is needed for every drone line used in a breeding program. Mating yards usually are not used for breeding stock, but rather for production queens where absolute control of matings is not quite as critical.

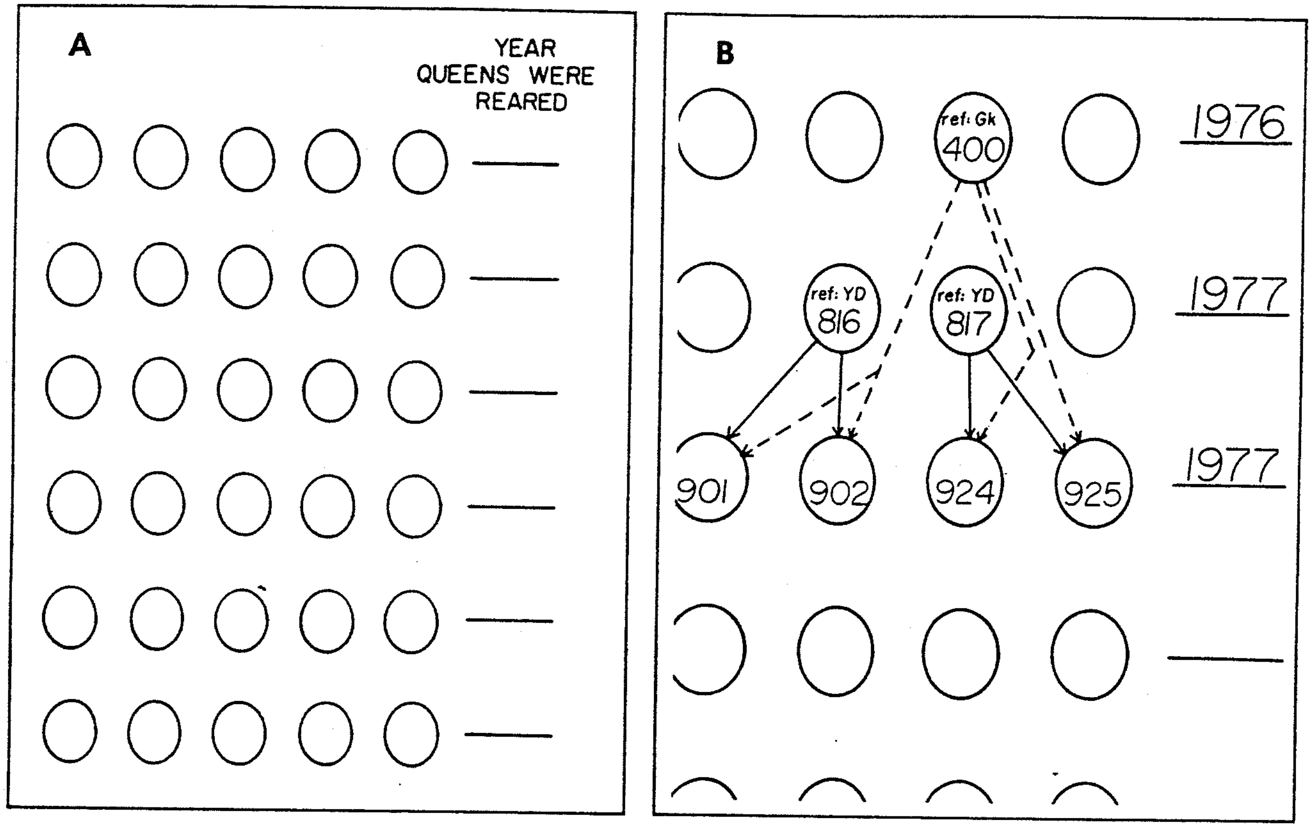

FIGURE 2.—A system for communicating bee pedigrees. *A* is a suggested format for a bee pedigree as printed on a sheet of paper; *B* uses the format of *A* to communicate some simple crosses. Each oval represents an individual queen and each line represents a gamete; dashed lines indicate male gametes (sperm) and solid lines indicate female gametes (eggs). The date on the right is the year that the queen or queens in that horizontal row were produced. Numbers in the ovals are for the breeder's convenience; they may be a queen number, a colony number, a breeding code, or a reference to another pedigree sheet. Any number of lines or gametes may generate from an oval, but each individual (oval) has either zero or two lines (one dashed and one solid) going to it. If a queen (oval) has no lines going to it, she is either unpedigreed or pedigreed on another sheet. Interpreting *B*: Drones from queen number 400 were mated to queens 816 and 817. Queen 816 was inseminated with semen from a single drone. Daughters subsequently were produced: 901 and 902 from 816, 924 and 925 from 817. The forked dashed line going to 901 and 902 indicates that the male gamete (sperm) going to queens 901 and 902 came from the same drone. Therefore, at least half the genes in queen 901 are identical with those of queen 902. The most common way a breeder would know this is a record of having inseminated queen 816 with a single drone. Queens 924 and 925 may or may not have a gamete in common.

Controlled breeding through instrumental insemination has been well established since 1947 and has solved the controlled-breeding problem for bee researchers, but commercial queen producers rely primarily on natural mating.

Recently, a few commercial queen producers have tried instrumental insemination for mass production of queens. Frequently, their reason for using it has been to eliminate mating nucs (small queen-mating colonies), rather than to make specific matings.

Instrumental insemination is, in itself, not a complicated process. Simply stated, it is a mechanical transfer of semen from the drone to the oviduct of the queen. This transfer can be made with any one of many designs of insemination stands and syringes available. All of them use carbon dioxide (CO_2) as an anesthetic to keep the queen still, use a device to hold the queen in position, and use some type of syringe to collect the semen and discharge it into the queen. Probably the most popular apparatus is that developed by Mackensen (fig. 3). Usually, one can become proficient at instrumental insemination, after practicing with 50 to 100 queens. Thereafter, the major problems and the major workload involve drone rearing, holding adult drones to maturity (about 2 weeks), queen storage, coordination of queen and drone production, queen introduction, and recordkeeping.

Instrumentally inseminated queens differ from naturally mated queens in their initial egg laying; instrumental insemination does not stimulate egg laying as does natural mating. If it were not for the carbon dioxide anesthetic given to queens during the following insemination, an instrumental-inseminated queen would begin laying eggs no sooner than a virgin queen that was not allowed to leave the colony to mate. The usual procedure is to render queens unconscious with CO_2 for 10 minutes on each of the 2 days following insemination.

Germplasm Storage

Germplasm is the hereditary material that can produce new individuals. In honey bees, this includes eggs, sperm, and tissue that can potentially produce eggs or sperm. Since every breeding program needs to keep certain stock for current and future use, the problem becomes one of either storing it (as with seeds on a shelf) or continually propagating it.

At the present time, honey bee germplasm is kept primarily through propagation. Thus, germplasm is usually in the form of mated queens—their ovaries and the sperm in their spermathecae. Alleles are lost gradually through inbreeding, so each generation of propagation reduces the variability of the germplasm slightly.

To avoid this loss and the labor involved in propagation, attempts have been made to store honey bee germplasm. Among the possible candidates for storage (eggs, larvae, pupae, virgin queens, sperm), sperm storage has been the most successful. Sperm stored less than 2 weeks at nonfreezing temperatures seems to be as viable as fresh sperm, but longer storage results in fewer sperm reaching the spermathecae. Although inferior inseminations result from sperm stored in liquid nitrogen (−196°C), nitrogen shows great promise for long-term storage where survival of the germplasm is the major concern.

Mutations

More than 30 specific visible mutations have been described in bees, and a number of these are maintained by research laboratories. Generally, these mutations produce a striking effect, and the majority have been easily observed by their discoverers. Many other mutations might occur in bees that also cause subtle changes yet to be observed. Known mutations affect the color, shape, and presence of eyes, the color and hairiness of bodies, the shape and size of wings, and nest-cleaning behavior.

Probably because of their distinctive appearance, most of the honey bee mutants thus far collected had variations in color of eyes. Various shades of white, tan, chartreuse, and red have been described and about 20 still are maintained. In addition to their value as curiosities, these mutants have value as scientific tools. For example, by studying various colors of eye mutants, the biochemical pathway for the production of eye pigments in honey bees was determined.

In addition to contributing to work on eye pigment biochemistry, mutants have been used as tools to investigate a variety of other questions. Resistance to American foulbrood, mating behavior, sex determination, pollination activity, fertilization technology, sperm storage, population dynamics, longevity, and bioacoustics all have been explored with experimental designs utilizing bees identifiably different because of mutations they carry. Because of this history of usefulness and further potential applications, it is desirable for the scientific community to maintain a number of mutations. Newly discovered mutations may have special applications in science; therefore, beekeepers could help by reporting mutations they observe to a research laboratory.

Most mutations are recessive. Mutations, therefore, are often first observed in drones, for drones are haploid and do not mask recessive genes. A mutation might occur in a single drone in a colony or in many drones.

Gene Pool

Across the world, bees are quite diverse. Time, mutations, and selection pressures have resulted in populations of bees called races, somewhat isolated from each other, that excel for various combinations of characteristics. These combinations of characteristics are finely tuned for survival in specific local environments. Worldwide, the races of bees form the gene pool or genetic base available to bee breeders for stock improvement.

Since North America and South America lacked native honey bees, European settlers imported them. Early importations were the brown bees common to northwestern Europe. Through time, beekeeping developed as an industry in North

America and beekeepers, happy with some characteristics of the European brown bee and unhappy with others, made further imports. Prominent among these imports were bees from other European areas. However, bees also were brought from Africa and Asia. The search by beekeepers for better bees led to a wide variety of genetic material being brought into America until 1922, when importing adult bees was banned to prevent the mite *Acarapis woodi* (Rennie), the cause of acarine disease, from entering the country. Eggs and semen, however, were still imported but at a much reduced rate. After 1976, importing any honey bee germplasm was banned by Public Law 94–319, except under authorization from the U.S. Department of Agriculture.

The bees presently in the United States are the result of free-mating crosses of the various imports. Most probably, racially "pure" stocks no longer exist in North America. Rather, this new genetic mix of bees can best be termed American. By the same token, exports from the United States and crossbreeding have influenced the nature of bees abroad.

The great virtue of our past imports is that we can breed highly desirable bees from the many varieties of stocks that we now have on the continent. Yet, some people still want to import more stock. Usually, the first thought that comes to mind when improved stock is desired is to import. It seems like a simple solution. Importing, however, should not be used as an easy substitute for a selection program. If used, importing would be only preliminary to a selection program, as an effort to expand the genetic base from which to select.

Thus, further stock imports are of questionable benefit. Such imports may result in acarine disease, may be themselves undesirable, or may combine with local stocks to produce undesirable hybrids. Many past imports of "select stock" proved to be poor or even undesirable in North America. In addition, imported bees may have originated from bees exported by American queen producers. For those interested in improving bee stock, therefore, it is probably best to select from the plentiful gene pool already available in North America.

Stock Improvement

Using improved stocks of bees is an effective way to improve the productivity of a beekeeping operation. Regardless of the stock of bees used, basic operational expenses will remain much the same.

Success in improving bee stocks is a reachable goal. As we have seen, there is great variation in bee stocks available to North American bee breeders. This variation is the raw material used by bee breeders. Working with the tool of selection, bee stocks can be molded to show high performance for desired characteristics.

Selection Methods

Describing the desired stock

The first task of a bee breeder is to describe in rather specific terms what characteristics are desired in the bee stock to be produced. Almost certainly a number of characteristics will be listed. Generally, desired characteristics will relate to the production needs of a group of beekeepers who are in similar localities or have similar needs. Desirable characteristics might include fast spring buildup, intensive honey production, frugal and strong overwintering ability, disease resistance, and good handling qualities. A different list might emphasize heat tolerance and pollination activity.

A knowledgeable bee breeder will be careful to be only as specific in his stock descriptions as good information permits. Unless scientific proof is developed to the contrary, physical characteristics such as color, size of bees, and shape of wings are poor choices. Generally, if such characteristics are important, they will be selected and improved automatically along with more general characteristics such as honey production or disease resistance.

A knowledgeable bee breeder also will set reasonable goals. Some characteristics, such as frugal use of winter stores and early strong buildup, are not likely to be highly compatible. A list of a few well-chosen characteristics is more likely to be achieved than a longer list.

Overall, there is a need in the beekeeping industry for a number of bee stocks, each having a collection of characteristics economically important to different segments of the diverse beekeeping community. No one bee stock can possibly be universally acceptable, and attempts to produce such a stock would prove fruitless. Thus, communication between an individual bee breeder and the beekeepers using the breeder's stock is important. This communication will help the breeder decide which characteristics to emphasize in the breeding program.

Measuring superior breeding stock

Once the breeding goal has been established by describing the desired stock, choices need to be made as to how the various characteristics will be measured. Although more precise ways to evaluate colonies may be devised in the future, at present the bee breeder must choose his stock from the on-site performance of colonies established in apiaries.

Test apiaries should be established with colonies arranged in an irregular pattern, with the colonies spaced as far apart as possible. Apiary sites that have trees, shrubs, or other such landmarks are valuable. These various precautions will tend to prevent the drifting of field bees. Other management procedures also should be reasonably uniform so that all colonies have an equal opportunity to perform. Management procedures should conform reasonably well to the management procedures used with production colonies.

Test colonies will be evaluated for the various characteristics to determine which colonies will be used as breeding stock. In all cases, beekeeping judgment will be brought to bear on the evaluation. The power to select more accurately the best breeders, however, will be enhanced if each colony is given a numerical score for each characteristic being evaluated. This will require the keeping of extensive records on colonies. Obviously deficient colonies can be left out of the recordkeeping to ease the load. Such records are particularly important when different evaluations, such as honey production and overwintering ability, are made at different times.

Once all the colonies have been evaluated, which will take 1 to 2 years, depending on the characteristics to be improved, breeder colonies can be chosen. The scores given to a single colony for all the various characteristics can be added to provide a single numerical score for the entire colony. Such scores can then be compared to select the best colonies available. More emphasis can be put on one or another characteristic by adjusting the scores given for that characteristic. For example, honey production may be scored on a scale of 0–20, while temper may be scored on a scale of 0–10. This arrangement would be used if honey production was considered twice as important as temper.

Breeding Methods

Line-breeding

The common method of breeding practiced by queen breeders is known as line-breeding. It can be defined as breeding and selecting within a relatively small closed population. The bee breeders' colonies constitute such a population to the extent that mismatings with drones outside their stock do not take place.

The general procedure in line-breeding is to rear queens from the best colonies. These queens are both sold as production queens and used to requeen the bee breeder's test colonies. The queens are allowed to mate with the drones present in the bee breeder's outfit at the time the queens are reared.

A number of variations can be made on this general procedure which would be of benefit. General control of drone brood in the majority of colonies, coupled with purposeful propagation of drones in a good number of more exceptional colonies, would improve the selection progress by controlling, to a limited degree, the male parentage of the stock. Of course, this procedure is used with the best success in areas where mating yards can be reasonably isolated.

In line-breeding, some inbreeding is inevitable. Its main effects are (1) fixation of characteristics so rapidly that effectiveness of selection for good qualities is reduced, (2) the stock loses vigor as a general consequence of inbreeding, and (3) the poor brood pattern from homozygous sex alleles. These effects can be lessened by using as many breeding individuals as possible for every generation.

To keep inbreeding at a minimum, one should rear queens from as large a number of outstanding queens as possible and requeen all the field colonies with equal numbers of queens from all the breeders. Each group of queen progeny is then considered a queen line and each year, after testing, at least one queen in each line is used as a grafting mother.

Despite these several precautions against inbreeding, stock may begin to show a spotty brood pattern and other symptoms of inbreeding. When this occurs, new stock must be brought into the operation. At least 10 virgin queens from each of several promising stocks should be mated with drones of the declining stock and established in apiaries outside the mating range of the beekeeper's queen-mating yards. They should be evaluated there to determine which stock(s) combine best with the deteriorating stock. Once this evaluation is made, the preferred stocks can be established as new queen lines.

In the 1930's, a 4-year selection project using simple line-breeding resulted in an increase in honey production from 148 to 398 pounds per colony. Two important features of this project were culling the poorer queens and grafting from the best queens.

Hybrid breeding

When inbred lines or races of bees are crossed, the hybrid progeny often are superior to either parent for one or many traits. This phenomenon is called *hybrid vigor* or *heterosis*. Hybrid bees have more heterozygosity in their genome than do inbred or line-bred bees. This heterozygosity is thought to be the basis for hybrid vigor.

Hybrid-breeding programs in bees are considerably more complicated than line-breeding programs. At the very least, three inbred lines must be combined so that both queens and their worker daughters are hybrids. An inbred queen mated to inbred drones will produce hybrid workers. However, the egg-laying qualities of the inbred queen probably would be inadequate. Therefore, there is a need to mate hybrid queens to inbred drones so that both queens and workers in production colonies are hybrids.

Four-line hybrids also are possible and commercially available. Such a hybrid may involve lines 1, 2, 3, and 4 and could be combined in the following way: An inbred queen of line 3 artificially mated to drones of line 4 is used as a grafting queen to produce hybrid (3 × 4) queens. These are allowed to mate naturally and are used to produce drones. Queens of line 1 are then mated to drones of line 2 and hybrid virgin queens (1 × 2) are reared from the mating. Production queens are produced from a cross of virgin queens (1 × 2) mated to the drone progeny from the 3 × 4 queens. Colonies produced by this cross will be headed by two-way hybrid queens, which will be uniform in appearance, whereas the worker bees will be four-way hybrids and variable in appearance, unless the color markings of the parent lines are very similar.

Comparative tests of hybrids have shown their superiority. Increased productivity of 34 to 50 percent over the average of line-bred strains has been reported. Segregation and random mating in the generations following hybridization are likely to result in queens that are no better than the average supersedure queen. Hybrids are an end product, and to make best use of them it is necessary to requeen every year.

Whatever the specific choice of breeding scheme, hybrid breeding requires the use of instrumental insemination and careful recordkeeping. As a consequence, few bee breeders have undertaken the entire operation of a hybrid program. However, many have become involved as producers of hybrid queens with the breeding stock supplied by an outside source.

ACKNOWLEDGMENT

We thank Dadant & Sons, Hamilton, Ill., and G. H. Cale and W. C. Rothenbuhler for permission to modify and use figure 1, which appeared in *The Hive and the Honey Bee*. James Baxter, biological technician in our laboratory, prepared figure 1.

References

CALE, G. H., JR., and W. C. ROTHENBUHLER.
1975. GENETICS AND BREEDING OF THE HONEY BEE. *In* The Hive and the Honey Bee, p. 157–184. Dadant & Sons (ed.) Journal Printing Co., Carthage, Ill.

DZIERZON, J.
1845. GUTACHTEN UBER DIE VON HR. DIREKTOR STÖHR IM ERSTEN UND ZWEITEN KAPITEL DES GENERAL–GUTACHTENS AUFGESTELLTEN FRAGEN. Bienen-Zeitung (Eichstätt) 1:109–113, 119–121.

LAIDLAW, H. H., JR.
1977. INSTRUMENTAL INSEMINATION OF HONEY BEE QUEENS. *In* Pictorial Instructional Manual. 144 p. Dadant & Sons, Inc. Journal Printing Co., Carthage, Ill.

MACKENSEN, O.
1951. VIABILITY AND SEX DETERMINATION IN THE HONEY BEE ("APIS MELLIFICA" L.). Genetics 36(5):500–509.

——— and K. W. TUCKER.
1970. INSTRUMENTAL INSEMINATION OF QUEEN BEES. U.S. Department of Agriculture, Agriculture Handbook No. 390, 28 p.

POLHEMUS, M. S., J. L. LUSH, and W. C. ROTHENBUHLER.
1950. MATING SYSTEMS IN HONEY BEES. Journal of Heredity 61(6): 151–155.

RINDERER, T. E.
1977. A NEW APPROACH TO HONEY BEE BREEDING AT THE BATON ROUGE USDA LABORATORY. American Bee Journal 117(3):146–147.

ROTHENBUHLER, W. C., J. M. KULINCEVIC, and W. E. KERR.
1968. BEE GENETICS. Annual Review of Genetics 2:413–438.