

CLOSED POPULATION HONEYBEE BREEDING

by ROBERT E. PAGE, JR AND HARRY H. LAIDLAW, JR

The North Central States Bee Research Facility, United States Department of Agriculture, Agricultural Research Service, Russell Laboratories, Room 436, University of Wisconsin, Madison, WI 53706, and Department of Entomology, University of California, Davis, CA 95616

Introduction

This article reviews methods of honeybee breeding and outlines how the genetics of sex determination in the honeybee have affected the success of breeding programmes. Recent computer simulations of bee-breeding programmes are described.

Traditionally, honeybee breeders have selected either annually or biennially a few superior queens to produce virgin queens for the following generation. Virgin queens produced from queen mother colonies are placed in mating nuclei in apiaries where they are either accessible during their mating flights to drones from drone mother colonies or they mate with drones from feral colonies. Improvements in commercial honeybee stocks from this method of breeding have been limited by the uncertain origin of the drones mating with virgin queens. It is, however, extremely difficult to isolate completely and/or saturate a mating area with drones of selected stock because of the mating behaviour of the honeybee.

Control over matings was gained by instrumental insemination and with its development bee breeders anticipated vast improvements in the performance of their bees. However, a new and severe problem became apparent: honeybees have a kind of self-incompatibility arising from the genic mechanism of sex determination. With inbreeding, this mechanism causes a commercially unacceptable reduction of brood viability within colonies (a condition known as "shot brood"). This condition contributes to the difficulty of evaluating queens, and to the perpetuation of lines of bees during the development and maintenance of a breeding programme.

The first directed use of instrumental insemination was to form inbred lines and to cross them to obtain specific-combining hybrids. (Specific-combining hybrids are hybrids from crosses of specific inbred lines. The individual lines may themselves have poor qualities but in specific combination with each other have good qualities. Some inbred lines may have good combining abilities and do well with a large number of inbred lines i.e. they have general combining ability. Inbred-hybrid breeding is a system of inbreeding lines then crossing those lines to produce hybrids.) In 1943, the United States Department of Agriculture Laboratories in Baton Rouge, Louisiana and Madison, Wisconsin, undertook a cooperative project in hybrid breeding and stock testing using instrumental insemination to control matings. Some, but not all hybrids produced by this programme were superior to open-mated stocks.

Many difficulties plagued the inbred-hybrid programmes and prevented their adoption by commercial bee breeders. These problems involved the unsuitability of the commercial hybrid queens as breeders because of the lack of uniformity of daughter

colonies and because the bee breeders were restricted to maintaining lines with low viability (a consequence of inbreeding). Producing and testing sufficient lines of bees to identify truly superior ones for combination and progressive development was expensive because lines were easily lost and were often irreplaceable. Consequently, the inbred-hybrid breeding programmes decreased in popularity and are now pursued by few bee breeders. This has prompted a reinvestigation of alternative conventional methods of mass selection and line breeding (other than inbred-hybrid breeding) within genetically closed populations, previously abandoned because the population genetics of the mechanism of sex determination were poorly understood^{12, 13, 17, 15, 16}.

Sex determination in honeybees

Sex in honeybees is determined by a single gene of which there are several different forms (alleles) in any population. Genes are incorporated in nuclear bodies called chromosomes, which are organized into sets that consist of one of each kind of chromosome. The sex gene occupies a particular place on one chromosome. Workers and queens (females) have two complete sets of chromosomes (are diploid) and thus have two sex genes, one in each chromosome set. Normal drones (males) arising from the unfertilized eggs of the queen have just one set of chromosomes (are haploid) and therefore one sex gene. To be female, a diploid individual must have two different forms of the sex gene (one on each set of chromosomes); individuals having the same allele of the sex gene on both sets of chromosomes develop into abnormal diploid males^{9, 10} which are effectively inviable, since workers detect and consume them shortly after they hatch from the egg^{18, 19}.

Number of sex alleles in honeybee populations

Honeybee populations have between 6 and 19 sex alleles^{1, 4, 6, 10, 20}. The number of different sex alleles found in a breeding population is affected by the size of the breeding population and by the rate of introduction of new alleles into the population, either from new breeding individuals or through chance mutation of the sex gene in breeding individuals (an insignificant consideration for commercial programmes). A common misconception is to regard the different sex alleles of the genic sex determination system as lethal genes because of the fate of abnormal diploid males.

Normally it is desirable to eliminate genes with lethal effects from a breeding population; however, for honeybees the goal of the breeder is to keep as many of the different alleles of the sex gene as possible in the breeding population. This can be illustrated by considering the case where there are just 4 different alleles of the sex gene. If all are represented equally in the population, each allele will represent 25% of all the sex genes. Since queens must have two different kinds of sex gene, and since each male has just one kind (because males are haploid), each time a queen mates with a drone there is a 50% chance that the drone and queen have an identical sex allele. If this occurs, half the brood from this mating will be inviable (see Fig. 1), and so overall the expected inviable brood from a queen in a population with 4 alleles will be 25%. However, if there are 10 different alleles of the sex gene, all represented equally, in the population, each will account for 10% of all the sex genes. Thus for each mating there will be only a 20% chance of the drone and queen sharing an identical sex allele. Since half the brood from such a mating will be inviable, the expected inviable brood from a queen in a population

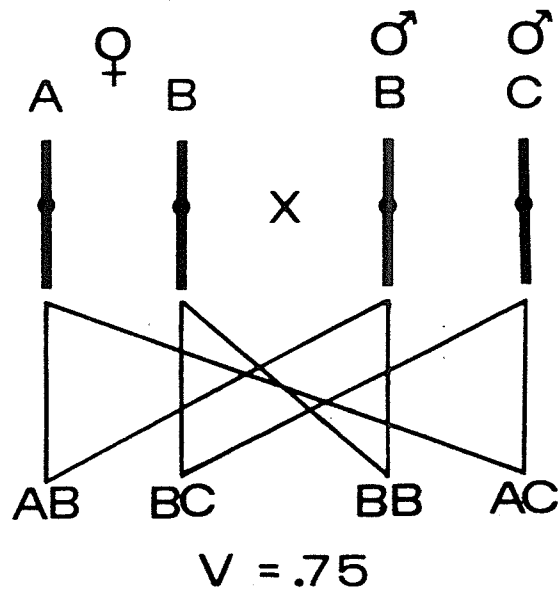
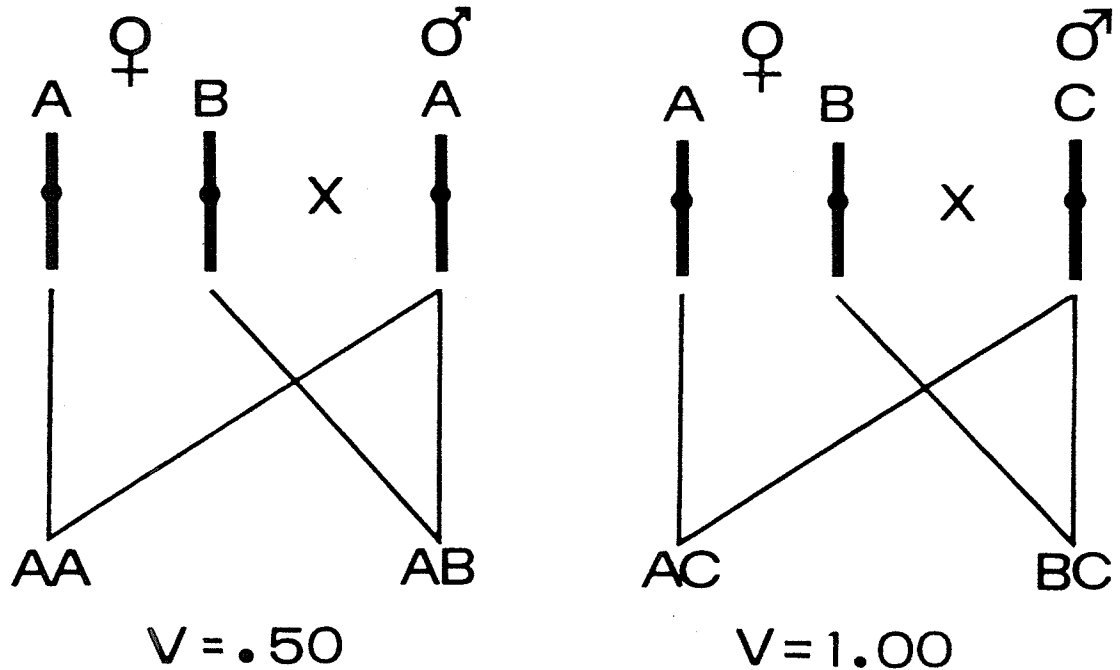


Fig. 1. (*top left*) A queen with sex alleles A and B is mated to a single male with an A type sex allele. The queen produces eggs each with a single sex allele; each allele has an equal chance of being represented in each egg. Sperm all have type A alleles. The expected genotypes of offspring are AA and AB in equal proportions. AA individuals develop into diploid males and are effectively inviable, therefore the expected proportion of viable (V) offspring from this mating is .50.

Fig. 2. (*top right*) As Fig. 1 except the male has an allele (C) different from either of the queen's, hence no diploid males are produced.

Fig. 3. (*bottom*) A queen is mated to one male with a sex allele like one of hers (B) and a second male with a sex allele different (C) from either of hers. Assuming the queen uses sperm of each male equally, 25% diploid males (BB individuals) are produced; ($V = .75$).

with 10 alleles of the sex gene will be only 10%. Therefore, the more alleles there are of the sex gene, the higher the expected brood viability within a population, because in a population with 10 alleles a queen would produce on average 90% viable brood compared with only 75% if there were only 4 alleles.

The effect of the number of queen matings on brood viability

The number of matings of each queen affects the size of the effective breeding population by increasing or decreasing the number of males represented²¹, and affects the distribution of brood viabilities among colonies within populations.

The increase in the effective size of the breeding population is not linear with an increase in the number of drones mating, if the number of queens is held constant¹³. The increase is also non-linear if the number of queens is increased while holding the number of drones constant¹³. Although the number of queens has a greater effect than the number of drones, the effective breeding population size is ultimately influenced most by whichever sex is in short supply in the population.

Increasing the number of matings per queen reduces the variation in brood viability between colonies caused by the genic mechanism of sex determination. If queens mate just once, colonies will have either 50% viable brood (if the drone has a sex allele like one of the queen's, see Fig. 1) or 100% viable brood (if the drone's sex allele is different from both sex alleles of the queen (see Fig. 2)). If a queen mates with two drones, each contributing the same number of equally viable spermatozoa, and if these mix randomly within the spermatheca of the queen⁸ then there are three possible classes of colonies: (1) 50% viability (both drones have alleles matching those of the queen); (2) 75% viability (one drone matches, see Fig. 3); and (3) 100% viability (neither drone matches the queen). With more matings there are more classes, and the viability of each colony is more likely to be close to the average for the population. If queens mate randomly (i.e. without selecting drones with a particular allele of the sex gene) the average viability over the whole population will depend on the number and frequency of different sex alleles. The variance in viability between colonies therefore depends on the number and frequency of sex alleles, and also on the number of matings of each queen.

Closed population breeding programmes

Closed populations are free from the uncontrolled introduction of genetic material. Successful closed population programmes have been responsible in plant and animal breeding for the improved commercial value of many economically important stocks. However, honeybee breeders have been restricted by the difficulties caused by the mechanism of sex determination. The principal obstacle to bee breeders in using closed populations was that smaller populations tend to have fewer sex alleles and, as described above, brood viabilities are lower. The paradox for honeybee breeders was how to maintain high brood viability under controlled mating, i.e. to determine the number of colonies needed to maintain sufficient sex alleles to ensure high brood viability, while remaining small enough to be economically manageable.

Recent work on the population genetics of sex determination^{20, 22, 2} and computer simulation studies^{5, 17, 13, 14, 12, 15, 16} have identified how many colonies are needed for successful breeding programmes and have also provided guides to the methods of queen

and drone selection, sperm usage, the introduction of new sex alleles, and population structure.

Throughout the following discussions a brood viability of 85% is used as the minimum which would be commercially acceptable. In the absence of a universally accepted minimum, this value has been widely used since its initial adoption (in 1982)¹⁷.

Computer simulation

Computer simulation studies have employed four basic breeding strategies: (1) random selection of queens; (2) queen supersedure; (3) top crossing; (4) selection for solid brood. In all four, the same breeder queens serve as both queen and drone mothers. It is assumed that breeder queens (i.e. colonies) making up the initial closed population are selected randomly from a very large base population of colonies in which 12 sex alleles (a likely value) occur at equal frequencies. In each generation, a large number of virgin queens and drones are produced from each selected colony. For strategies 1, 2 and 3, ten drones are randomly selected from the drone pool available in that generation, to inseminate the daughter queens produced. The drone pool is composed of a large number of drones assuming equal contributions from each breeder queen. For strategy 4 (selection for solid brood) the semen of a large number of drones, again representing equal contributions from each breeder queen, is pooled and homogenized³. New closed population breeder queens are selected from each generation of inseminated daughters to replace the previous generation of breeder queens.

1. *Random selection of queens:* New breeder queens are randomly selected from each generation of daughter queens produced. This does not mean that economic criteria cannot be used when selecting queens; rather that selection is random with respect to the sex alleles of each queen. Fig. 4 shows that under this system about 50 breeder queens must be selected from each generation to maintain a 95% probability of having at least 85% viable brood over 20 generations (which might reasonably be the expected life of a breeding programme).

Occasionally a queen will produce a disproportionate number of superior daughters. However, if too many daughter queens are selected from a single queen mother too frequently, sex alleles will be lost more rapidly in the closed population.

2. *Queen supersedure:* With this system of queen selection, each breeder queen is replaced each generation by one of her superior performing daughters, so that the effective size of the breeding population is increased (because of a reduction in the rate of loss of sex alleles in queens) and consequently sex alleles are lost more slowly. Using this system about 35 queens yield the same result as 50 queens under the random selection system (see Fig. 5). However, it should be emphasized that selected characteristics will be gained more slowly because of the obligate selection of a single daughter from each breeder queen.

3. *Top crossing:* Top crossing is a method commonly used by plant and animal breeders where a superior performing pollen plant or sire is identified and crossed to a large number of individuals within the breeding population. This is a valuable method for rapidly increasing the frequency of desirable characteristics within breeding populations.

6

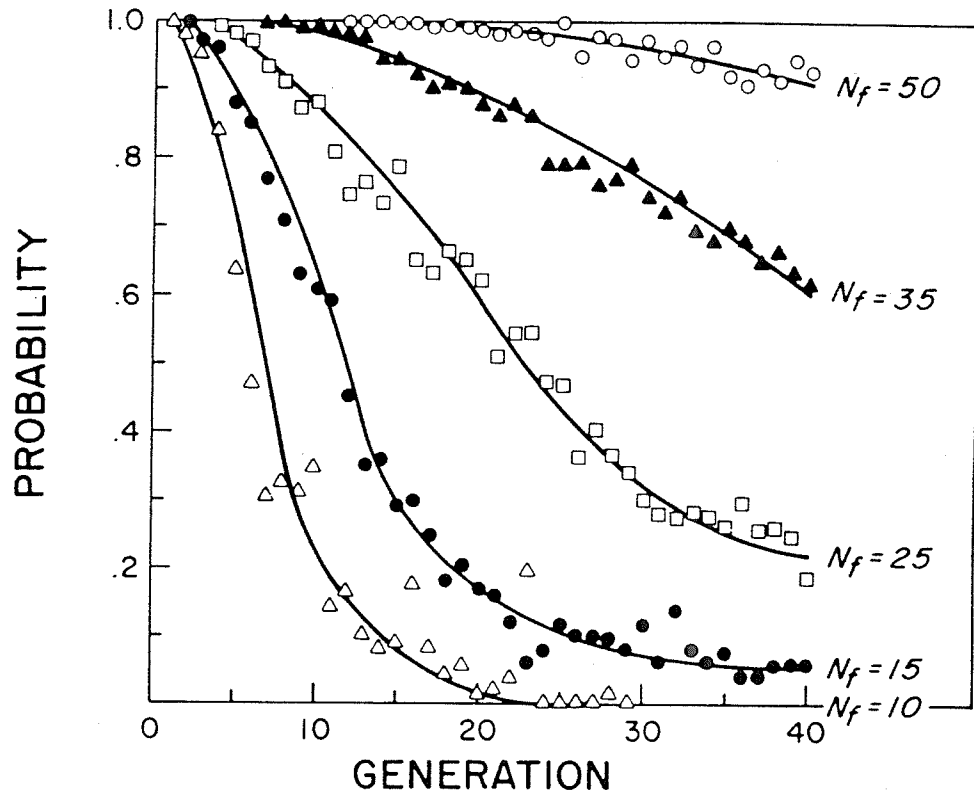


Fig. 4. The probability that the average brood viability in closed populations of 10, 15, 25, 35, or 50 breeder queens will be *at least* 85% using the random queen selection system. Results were determined by computer simulation (redrawn from Page, Laidlaw, and Erickson, 1983).

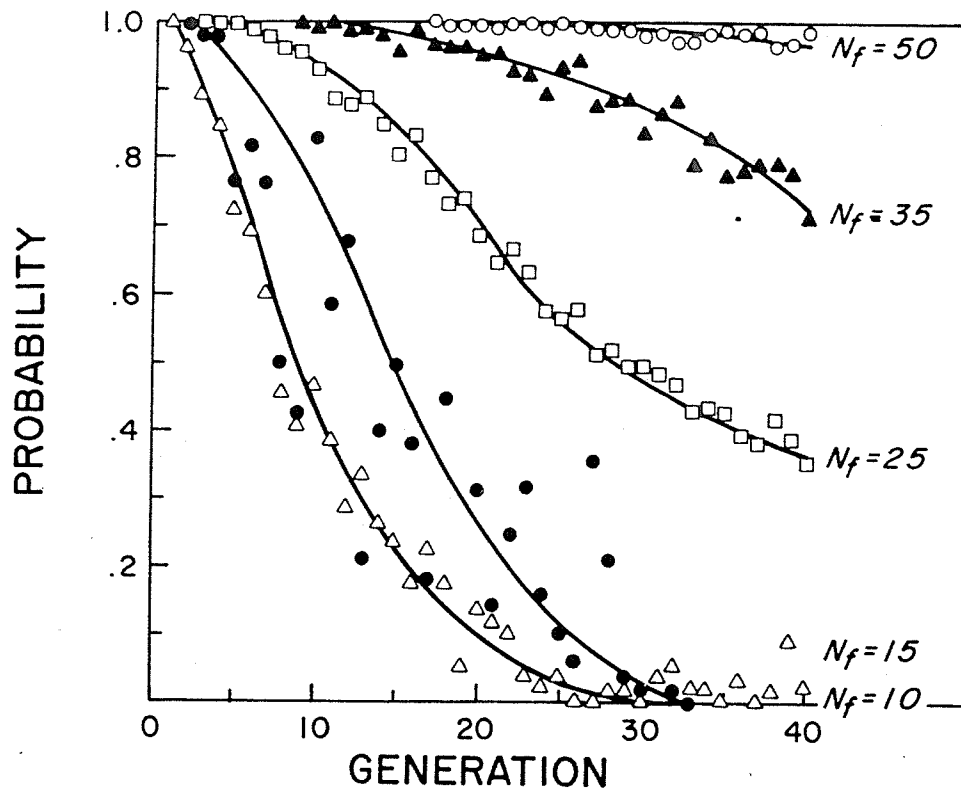


Fig. 5. As Fig. 4 except using the queen supersedure system (redrawn from Page, Laidlaw, and Erickson, 1983).

For honeybees, this is equivalent to identifying a superior queen (either from within or from outside the closed population) to be the drone mother for up to 100% of the inseminations of daughter queens within the closed population.

Drones from a top cross queen may be used in two ways: (1) up to 100% of all daughter queens produced from the closed population breeders may be inseminated exclusively with their semen; or (2) the drones from the top cross queen can be mixed in proportions of up to 100% with drones from the remaining closed population breeder queens, and then drones are drawn randomly from this mixed pool to inseminate all daughter queens produced. A given queen, however, should be used only once as a top cross parent. These top cross methods may be superimposed upon the random and the queen supersedure selection methods described above.

Results of computer simulations¹⁶ show that pooling drones from a top cross parent queen* with drones from all other closed population breeder queens in a predetermined proportion (preferably less than 75%) results in a slower loss of brood viability than when a designated proportion of virgin queens are inseminated exclusively with drones from the top cross parent. Top crossing should not be employed in populations with fewer than 50 breeder queens nor should a given population be top crossed more than 3 times, assuming top crossing with 75% contribution every other generation. Top crossing in consecutive generations results in faster loss of viability than when generations are skipped. Queen supersedure selection results in a slower loss of brood viability with top crossing. Selecting a top cross queen from outside the closed population results in a slower loss of sex alleles than selecting one from inside; however, if the top cross queen is selected from outside, the population is no longer technically "closed".

4. *Selection for brood solidness:* Kubasek⁵ used computer simulation to study the maintenance of sex alleles and brood viability in closed populations when breeder queens are selected for brood solidness. He assumed that the semen of all drones contributing spermatozoa to queens is pooled and completely mixed (homogenized) before insemination. This results effectively in each queen being tested against the genetic background of the whole population¹⁴. Queens with a rare sex allele (due to genetic drift) will have an above average brood viability since there is a below average chance of any given sperm with which she is inseminated carrying an allele identical to one of hers. Using a closed population of 50 breeder queens for his simulations, Kubasek found that selection for high brood solidness can be effective in preventing the loss of any sex alleles from the population. Kubasek also showed that selecting other characters such as honey yield did not significantly increase the rate of loss of sex alleles in closed populations. However, care should be taken when interpreting these results because of the simplistic model used and the underlying assumptions. Page and Laidlaw¹⁴ point out that selecting other characteristics should result in a greater loss of sex alleles under the random selection system because superior queens will be over-represented. Such selection should have less effect on the loss of sex alleles if queen supersedure is used.

*A 'top cross' is one of the breeder queens of the closed population. Her sons are pooled with drones from all of the rest of the closed population breeder queens.

Population structure

A closed population may be subdivided into two or more smaller parts with each subdivision distinct from the others—this has both advantages and disadvantages over maintaining a single large population. Each subpopulation will have a lower average viability than a single large population but the total number of sex alleles in the subpopulations is expected to equal that of a single large one. The advantage comes when matings of queens are limited to drones from different subpopulations. When this occurs, the expected average brood viability of these queens is greater than the average of their subpopulations because of the variance of sex allele frequencies between the subpopulations.

Introducing new sex alleles

If brood viability decreases unacceptably, it should be possible to increase it again by introducing new or rare sex alleles into the closed population. The introduction of undesirable genes should be minimized by only using queens of superior stock. Queens outside the closed population can be tested for rare or missing alleles by inseminating them with pooled and homogenized semen from drones representing all queens in the closed population. Queens with different sex alleles from those in the closed population will have significantly higher brood viabilities (approaching 100%) than those within the population. These queens can be used to supply up to about 25% of both virgin queens and drones to the closed population.

Pooled semen

Laidlaw⁷, Page and Laidlaw¹⁴, and Moritz¹¹ suggested the use of pooled homogenized semen in closed population breeding programmes. Queens can be mated to a very large proportion of the gene pool of the previous generation by inseminating them with pooled, homogenized semen from a large number of males, representing equal contributions from all the closed population breeder queens. This method should minimize variance in brood viability among colonies and enhance the breeder's ability to select superior queens without the confounding problem of high variance in brood viability. Subsequent differences in colony performance should reflect differences in the general combining abilities of the genotypes of the queens.

Another benefit of pooled, homogenized semen is its affect on the within-family genetic variance. As stated above, queen supersedure has the disadvantage of slowing bee improvement because of the obligate selection of each generation of a single daughter per breeder queen. With queen supersedure, the rate of improvement is determined by the amount of genetic variance within colonies since a single daughter is selected from each colony as a replacement for her mother. With pooled, homogenized semen, the within-family genetic variance can be approximately 75% that of the entire population (compared to 25% for single-drone inseminations), which greatly enhances the potential for selective improvement.

Commercial applications

Maintaining and testing a large number of closed population breeder queens can be time consuming and costly, and so queen breeders interested in this method could benefit by

cooperation. Each breeder could maintain a separate subpopulation of perhaps 10 or more colonies, and select the best stock (either queens or semen) within their subpopulation to exchange with other cooperators for upgrading and cross breeding. Daughters of these queens could then be mated to other stock, resulting in high brood viability and commercially valuable production queens. This kind of breeding programme would require a consensus of which characteristics were important and how they would be selected. Allele restoration, if needed, could be accomplished by top crossing or queen exchange between subpopulations.

Closed populations can preserve germ plasm and provide "sanctuaries" for desirable characteristics that can be introduced into undesirable feral populations. They may also preserve populations of bees with specific race characteristics if they are threatened by commercialization. A queen supersedure method of replacement is recommended for this since it results in the slowest loss of genetic material for a given number of queens.

A model programme

A model closed population breeding programme has been established by the United States Department of Agriculture, Bee Research Unit, at Madison, Wisconsin. The purpose of this programme is to develop the management practices necessary for successful implementation of commercial breeding programmes. It is also designed to test some of the theoretical aspects outlined above. It should be emphasized that much of the programme discussed above is based on genetic theory and has had only pilot testing, but it is hoped that this work will lead to a revitalization of interest in bee breeding and put us back in search of a better honeybee.

References

1. ADAMS, J.; ROTHMAN, E. D.; KERR, W. E.; PAULINO, Z. L. (1977) Estimation of number of sex alleles and queen matings from diploid male frequencies in a population of *Apis mellifera*. *Genetics* 86 : 583-596
2. CORNUET, J. M.; ARIES, F. (1980) Number of sex alleles in a sample of honeybee colonies. *Apidologie* 11 : 87-93
3. KAFTANOGLU, O.; PENG, Y.-S. (1980) A washing technique for collection of honeybee semen. *J. apic. Res.* 19 : 205-211
4. KERR, W. E. (1967) Multiple alleles and genetic load in bees. *J. apic. Res.* 6 : 61-64
5. KUBASEK, K. J. (1980) Selection for increased number of sex alleles in closed populations of the honey bee. An investigation via computer simulation. *Unpublished MS thesis : Louisiana State University*
6. LAIDLAW, H. H.; GOMES, F. P.; KERR, W. E. (1956) Estimations on the number of lethal alleles in a panmictic population of *Apis mellifera*. *Genetics* 41 : 179-188
7. LAIDLAW, H. H. (1981) [Honey bee genetics and its application to pollinator breeding.] *Honeybee Sci.* 2 : 1-4 *In Japanese*
8. LAIDLAW, H. H.; PAGE, R. E. (1984) Polyandry in honeybees (*Apis mellifera* L.) : sperm utilization and intra-colony genetic relationships. *Genetics* 108 : 985-997
9. MACKENSEN, O. (1951) Viability and sex determination in the honey bee. *Genetics* 36 : 500-509
10. ——— (1955) Further studies on a lethal series in the honey bee. *J. Hered.* 46 : 72-74
11. MORITZ, R. F. A. (1983) Homogenous mixing of honeybee semen by centrifugation. *J. apic. Res.* 22 : 249-255
12. PAGE, R. E.; ERICKSON, E. H.; LAIDLAW, H. H. (1982) A closed population breeding program for honey bees. *Am. Bee J.* 122 : 350, 351, 354, 355

13. PAGE, R. E.; LAIDLAW, H. H. (1982) Closed population honeybee breeding. 1. Population genetics of sex determination. *J. apic. Res.* 21 : 30-37
14. ———(1982) Closed population honeybee breeding. 2. Comparative methods of stock maintenance and selective breeding. *J. apic. Res.* 21 : 38-44
15. PAGE, R. E.; LAIDLAW, H. H.; ERICKSON, E. H. (1983) Closed population honeybee breeding. 3. The distribution of sex alleles with gyne supersedure. *J. apic. Res.* 22 : 184-190
16. ———Closed population honeybee breeding. 4. The distribution of sex alleles with top crossing. *J. apic. Res. In press*
17. PAGE, R. E.; MARKS, R. W. (1982) The population genetics of sex determination in honey bees: random mating in closed populations. *Heredity* 48 : 263-270
18. WOYKE, J. (1963) Drone larvae from fertilized eggs of the honeybee. *J. apic. Res.* 2 : 19-24
19. ———(1963) What happens to diploid drone larvae in a honeybee colony. *J. apic. Res.* 2 : 73-75
20. ———(1976) Population genetics studies on sex alleles in the honeybee using the example of the Kangaroo Island bee sanctuary. *J. apic. Res.* 15 : 105-123
21. WRIGHT, S. (1933) Homozygosis and inbreeding. *Proc. natn. Acad. Sci. U.S.* 19 : 411-420
22. YOKOYAMA, S.; NEI, M. (1979) Population dynamics of sex-determining alleles in honey bees and self-incompatability alleles in plants. *Genetics* 91 : 609-626