GAMETE-BACKCROSS MATINGS IN THE HONEY BEE

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THE queen of the honey bee (*Apis mellifera* L.) lays both diploid and haploid eggs, both of which are fertile. The diploid egg develops into either a worker bee or another queen—both of which are female—while the haploid develops into a hemizygous drone (male). Within this drone several million sperm are formed, all of exactly identical genetic constitution, barring the possibility of rare gene or chromosomal mutational accidents occurring in the germ line. The drone bee, therefore, represents a considerable pool of identical gametes; the queen spermatheca serves as an ideal storage compartment for the preservation of these gametes. Development of a technique for repeated backcrossing to this identical gamete pool should have significant applications to bee breeding.

WRIGHT (1921) treated the degree of homozygosity that could be obtained by repeated mating back to a homozygous sire, showing that the mean percentage of heterozygosis would be halved each generation and giving the series ½, ¼, ⅛, etc. CROW and ROBERTS (1950) pointed out the theoretical possibility of using stored sperm from the queen spermatheca for subsequent matings with the daughter queen. They did not regard this idea as practical owing to the lack of developed technique at that time.

WATSON (1927) first demonstrated the possibility of using stored sperm from the queen spermatheca for subsequent matings with the daughter queen, and his feat was duplicated by United States Department of Agriculture technicians as reported by NOLAN (1937). Both used multiple drone sources, or mixed gametes.

Current studies by the authors make use of single-drone matings, using instrumental insemination techniques. During the course of these studies, more than 50 queen bees instrumentally inseminated with the sperm from only one drone each have been kept successfully in large colonies for more than six months. This work made it seem practical to consider using sperm stored in the queen spermatheca for subsequent matings. An additional study by the authors (CALE and GOWEN 1956) made it seem likely that this technique might lead to rapid formation of highly desirable inbred lines. These earlier data showed that additive genetic effects were clearly demonstrated in crosses between certain inbred lines of bees. This, coupled with the possibility of single-drone insemination with backcross to identical gametes, has led to the following study of the technique for multiple backcrosses to a single gamete.

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EXPERIMENTAL

Two inbred lines of honey bees were used. The theoretical coefficients of inbreeding were 88.7 percent for line H, and 98.5 percent for line A. Both lines have a high general combining ability, and the earlier work (1956) demonstrated that this additive type of gene action was twice as important as specific combining ability for honey yield.

Lines H and A were crossed and daughter queens were then allowed to fly and mate at random. These random-mated F_1 daughters became a source of drones—each drone hemizygous, but each different from the other in genetic constitution. When these HA drones were available, ten additional HA F_1 virgin queens were reared from the original cross. These F_1 queens were then instrumentally inseminated, each with the sperm of only one HA drone.

Within ten days all F_1 queens were laying, and ten daughter queens were then reared—one from each of the single drone-inseminated mothers. The mothers were killed; the sperm was removed from their spermatheca and placed in the median oviduct of the respective daughter. Nine of these ten inseminations were successful. The total time lapse for the cross and backcross to identical gamete was 58 days.

The next step involved rearing additional daughters—one from each of the nine successful previous inseminations—and transferring the sperm another time in an identical manner. Six of these inseminations were successful, with more than sufficient diploid progeny to perpetuate each as separate, highly inbred and highly homozygous lines. Total time lapse for the entire triple sperm usage was 82 days.

Some discussion of the technique for gamete recovery from the spermatheca is presented here as guidance for those who wish to use this method. Careful selection was made of the original drones used, with choice limited to those which were fully formed, free flying, vigorous, and which gave a visibly large amount of sperm when killed.

Semisterile techniques were employed to make the gamete transfers. Of course, the first gamete usage was from drone to queen, while both the second and third involved passage of sperm from the spermatheca of one queen to the spermatheca of another.

Equipment used involved the standard queen bee instrumental insemination instruments, a glass microscopic slide, small dissecting scissors, small tweezers, dissecting needles, and an insect pin. All instruments were sterilized in alcohol and then rinsed in sterile distilled water. As a further precaution, the hands of the operator as well as the laboratory table were sterilized with alcohol.

For the two gamete backcrosses, the queen bee from which the sperm was to be taken was first anesthetized with carbon dioxide gas and then killed by crushing the head and thorax. Dissecting scissors and tweezers were used to open the abdomen and remove the spermatheca, which was placed on the glass slide. A thin film of sterile water was placed on the slide to prevent the spermatheca from drying. The dissecting needles were then used to remove the tracheal network on the outer surface of the sac-like spermatheca. Removal of the trachea and subsequent operations were performed under a 16× binocular microscope.

The insect pin was used to puncture the spermatheca. Then the tip of the insemination microsyring was inserted in the punctured area of the spermatheca and the sperm was removed with the microsyringe. The virgin queen was then instrumentally inseminated using normal techniques.

After insemination, queens were kept in very small hive units (nuclei) and were allowed to

lay only a few eggs. Even after three sperm transfers, it was evident—by examination of the sperm content of the spermatheca—that enough of the original gamete material was present for at least one additional transfer.

DISCUSSION

The success of this study makes it seem feasible to test the possibilities of gamete-transfer matings in the honey bee in future breeding research. In this case, two inbred lines were crossed and six new inbred lines were then formed by the use of six different gametes through three generations each.

Female progeny of the first cross would carry 50 percent of the gamete in their genetic make-up, along with 25 percent original H chromosomal material and 25 percent original A chromosomal material. Progeny after the first gamete-backcross would carry an average of 75 percent of the gamete, along with 12.5 percent of H and 12.5 percent of A chromosomal material. After the second gamete-backcross, progeny would carry an average of 87.5 percent of the gamete, and 6.25 percent H and 6.25 percent A chromosomal material.

On the average, 50 percent of the remaining H chromosomal matter would be paired with H and 50 percent of the remaining A chromosomal matter would be paired with A. The balance of the H and A chromosomal material would be paired together. Thus, the approximate homozygosity in each line at the end of the gamete transfer was 96.5 percent. This breeding method can be a useful tool in forming newer inbred lines rapidly from a combination of two older, tested inbred lines—using only twelve weeks for inbred-line formation instead of seven or eight years.

Further use of gamete backcrossing could be made in the quick formation of numerous inbred lines from a selected phenotypically superior queen within a panmictic population. Each line formed would be different, since the queen would be heterozygous for many characteristics and each drone (gamete) used for mating would be genetically different.

SUMMARY

Ten queen bees were instrumentally inseminated, each with the sperm of a single drone. A daughter was then reared from each mating and sperm was transferred, using instruments, from the spermatheca of the mother to the median oviduct of the daughter. This process was then repeated for a second gamete-backcross. Six highly inbred lines (96.5 percent) were thus formed in a time lapse of only twelve weeks.—Two possibilities for future use are suggested: (1) a cross between two inbred lines that have been shown to have a high general combining ability, with subsequent use of the F_1 for gamete backcrossing and new line formation; (2) the formation of many inbred lines using a tested superior queen from a panmictic population. Both gamete-backcross methods will achieve a high percentage of homozygosity within a period of only twelve weeks, as compared to years of effort with more conventional breeding schemes.

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