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A STANDARD STOCK OF HONEYBEES*

WILLIAM C. ROBERTS

Bee Breeding and Stock Center Research, Agricultural Research Service, USDA, Rte 3, Box 82-B, Ben Hur Road, Baton Rouge, LA 70808, USA

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Summary

A system of mating used in establishing a standard stock of honeybees from 16 lines is outlined. Alternative modifications using 24 lines suggest possible methods of more efficiency in measuring the mathematical consequences of this system and in reducing the loss of heterozygosity. Some results are given, and the possible use of these systems to establish improved breeds of bees is suggested.

Introduction

In experiments designed to measure genetic differences between stocks, a standard population is essential. Such a population should have a mean and a variance which are subject only to environmental differences that are common to both the test and the control populations. Moreover, when the control population has a genetic mean and variance that differ little from generation to generation, long-term progress in breeding can be meaningfully assessed, and the control population becomes the standard by which other populations are judged. A standard stock is also essential for accurate measurements of environmental variables because, when genetic variance is known, then environmental variance can be measured.

Standard stocks are widely used in selection and nutritional studies of poultry, and a standard stock of mice is also widely used in medical research. However, these standards differ in one important aspect: the mice are hybrids between highly inbred lines (Green, 1962), whereas the poultry represent a genetically heterogenous line that is maintained by random matings within a closed population with a minimum increase in inbreeding in successive generations (Gowe et al, 1959).

In the honeybee (*Apis mellifera*) we have a need for a standard stock. A hybrid between inbred lines is possible, but the difficulties involved in producing and maintaining inbred lines—and the possibility of loss of one or more of these—suggested the need for a new method of producing a standard stock. The breeding method used with poultry was investigated and found not to be feasible, principally because of the adverse results of mating honeybees which have one or more sex alleles in common (Mackensen, 1951).

The honeybee colony is a social structure consisting of two interdependent generations. The queen is long-lived (average about 1 year), but her worker offspring are short-lived (6-8 weeks) and are continuously replaced by their younger sisters. When the queen is replaced, the colony is soon repopulated by worker offspring of the new queen. However, the males that mate with the new queen are haploids—"gametes with wings" produced by their mother—so, genetically, they belong to the preceding generation. Thus, in honeybees, brother-sister matings are genetically

* In co-operation with Louisiana State University.

equivalent to parent-offspring matings (Crow & Roberts, 1950). Since the queens normally mate with many drones, 6–10 or more (Taber, 1954), their female offspring may consist of several groups of full and half-sisters. Matings are accomplished in a few days, and sperm is stored in the queen for a year or more. Thus the effective breeding population within a small sub-group of honeybee colonies is probably similar to that described for snails (Murray, 1964).

Mackensen (1951) showed that the sex of honeybees is determined by genes at the X locus. The many alleles at this locus are designated X^a, X^b . . . etc. Females are heterozygous for any two of these alleles, and males are hemizygous for any one. Homozygosity produces males, but these are usually non-viable. Therefore natural selection in honeybees acts principally on the entire colony, so a queen that mates with any male having a sex allele in common with her produces many fertilized eggs that are "lethal". Obviously, a colony headed by such a queen is at a selective disadvantage.

The facts, therefore, indicate that if random matings within a closed population are used to maintain a standard stock of honeybees, some matings will occur between mates having common sex alleles. To decrease this probability in a closed breeding population, we prepared a circular breeding plan which selectively paired distantly related individuals.

Materials and Methods

At the Honey Bee Stock Center, various inbred lines derived from many sources are maintained. If a number of these are crossed and composited into a closed mating group, then selective matings in each generation based on least relationship could (*a*) keep inbreeding within the population to a low level, (*b*) maintain the large number of sex alleles necessary for good brood viability, and (*c*) maintain a large proportion of the genetic variability existent in the original population. A programme to investigate the consequences of such a system of bee breeding has been begun. For purposes of simplicity, we here group the parental lines used in this programme into two major categories—black and yellow body colour. The black (and mostly black) lines include stocks originating from various geographical regions (Caucasian, Carniolan, Rumanian, Greek, Anatolian, German); all the intermediate and mostly yellow lines probably have some of their inheritance from stock of Italian origin, but these are not of recent import, so they represent American selections generally designated as Italian bees.

In 1968, queens from four yellow lines and four black lines were instrumentally inseminated with semen from drones of unrelated lines (Fig. 1). We will not attempt to describe the inbred lines that entered this programme; however, the lines selected had desirable characteristics.

The 16 lines of bees resulting are here designated A, B, . . . S. Queens A and A′, B and B′, etc. are sisters. Queen A is shown as a hollow circle (o) from whom only female offspring are obtained; queen A′ is represented by a solid circle (●) whose drone offspring were used in matings. Solid lines represent female gametes (eggs), and broken lines represent male gametes (drones) produced by the originating queens.

After initial compositing from 24 queens, we retained only 16 in each succeeding generation for breeding purposes. These are shown as 1, 1′, 2, 2′, . . . 8, 8′. The daughter of queen 1 which is used as the source of queens is always designated 1. Queen 1′ serves as the source of drones (male gametes) only.

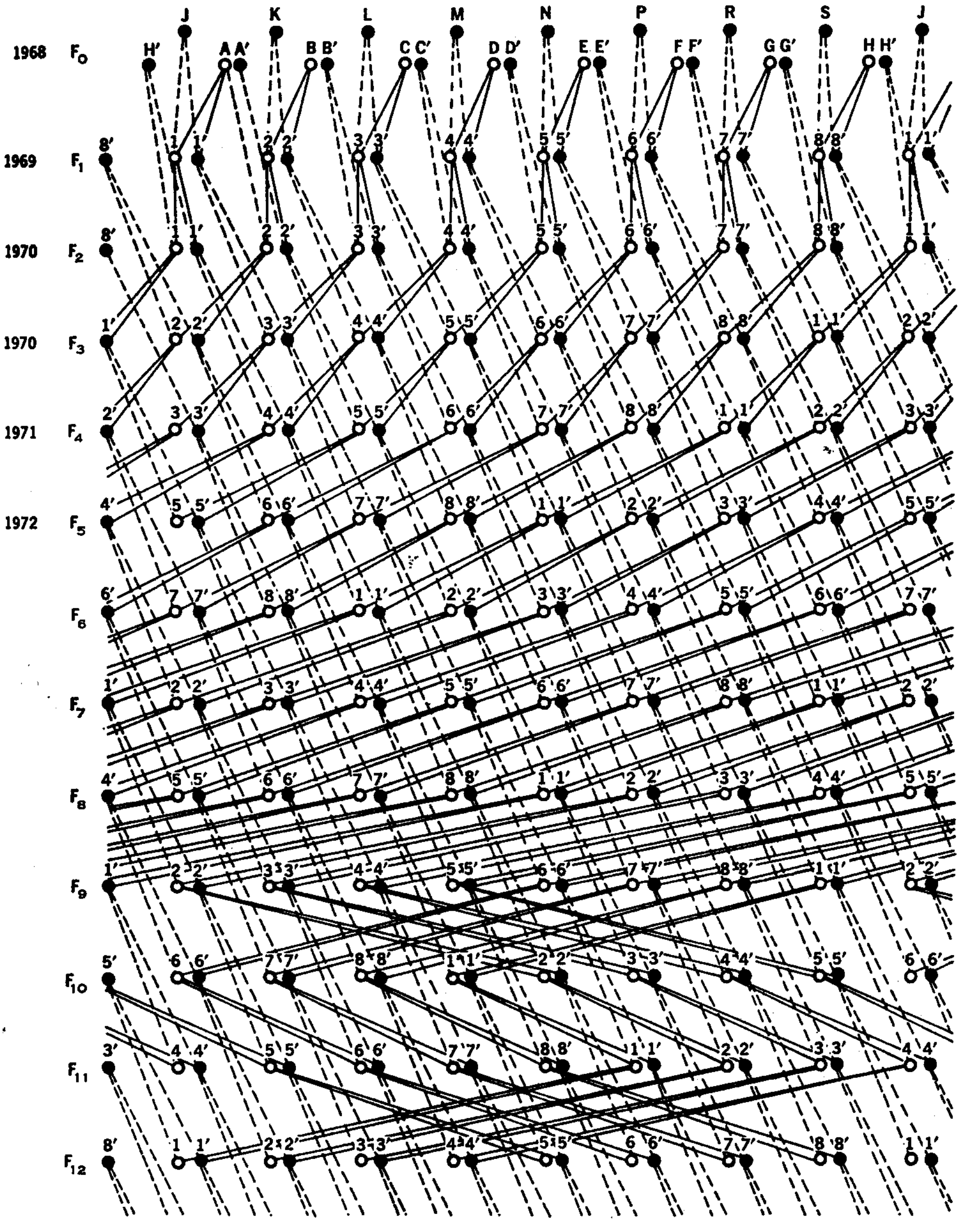

FIG. 1. Mating system for the standard stock of honeybees. Solid lines represent female gametes, and broken lines indicate male gametes.

As noted in Fig. 1, inbred queen H was mated to drones from an inbred queen of line S. Her daughter is designated subline 8; a daughter of line G queen is designated subline 7, etc. . . . A queen × J drone as 1. In 1969, daughters of queen 8 (HS hybrids) were mated to drones produced by inbred queen G′ (7). Likewise daughters of inbred queens G (7) (GR hybrids) were mated to drones produced by inbred queen F′ (6) . . . AJ queen × H′ drone. Two sister queens of each of the 8 sublines were used as breeders in each succeeding generation.

Fig. 1 shows that queens produced by subline 8 in the second generation (1970) were mated with drones from subline 6′, HSG queen × PF drone. The pedigree is drawn so that it can be looped into a cylinder for easier identification of the paths. The entire mating plan for each subline through 20 generations is shown in Table 1.

A minimum of 2 queens (colonies) was produced in each generation for each of the 8 sublines; the maximum may be 100 or more. Selection within sublines is limited by the number of queens (colonies) and the variability within the populations. Without selection, the mean variance of the entire population should be small between generations. In each generation, we select 2 queens from each of the 8 sublines. Their progeny are then mated in the predetermined manner.

We produce about 12 queens of each of the 8 sublines each year for maintenance of the stock, and each year our selections of breeding queens for the next generation are based on two characteristics: (*a*) high viability of brood to ensure unlike sex alleles, and (*b*) gross performance (temper) with a view to obtaining "desirable" colonies and rejecting the "undesirable" colonies. Other variables are ignored to assure heterogeneity in the population.

Results and Discussion

After six generations, the standard stock sublines are becoming similar in colour markings, and brood viability remains high in all lines—an indication of many sex alleles in the population. Thus, two of our objectives appear to be attainable, but the third cannot be estimated with accuracy. The mating system utilized multiple drone inseminations, each queen being inseminated with semen obtained from 6–8 drones. However, these drones were brothers, and each represented one gamete from the mother queen. Since all the sperms produced by an individual drone are genetically alike, the two selected daughters of a single queen may either be offspring of a single gamete (drone) or different gametes (brother drones) from the same parent. The probable loss of heterozygosity could, therefore, not be calculated with accuracy. To solve this problem, we have now designed a slightly altered mating plan (Fig. 2), and it is being used in another programme to develop a "breed" of bees.

In the second programme, we wished to establish a standard "Italian breed" by compositing the genomes from the best 24 Italian lines of bees available in the United States. One mated queen of each line was obtained; they were designated A, B, . . . X, Y, Z as shown in Fig. 2. After the initial compositing and the formation of eight pairs of sublines, each queen selected as a breeder for the next generation serves as a source of both male and female gametes. Multiple drone inseminations are made, but only one female offspring of each queen is allowed to produce offspring for the next generation, to eliminate the possibility of having two breeding individuals originating from a single gamete. This closed breeding system is similar to the circular breeding plan described by Kimura and Crow (1963) except that each individual queen leaves two offspring that are half sibs.

The system of mating shown in Fig. 2 comprises two parallel lines utilizing only 8

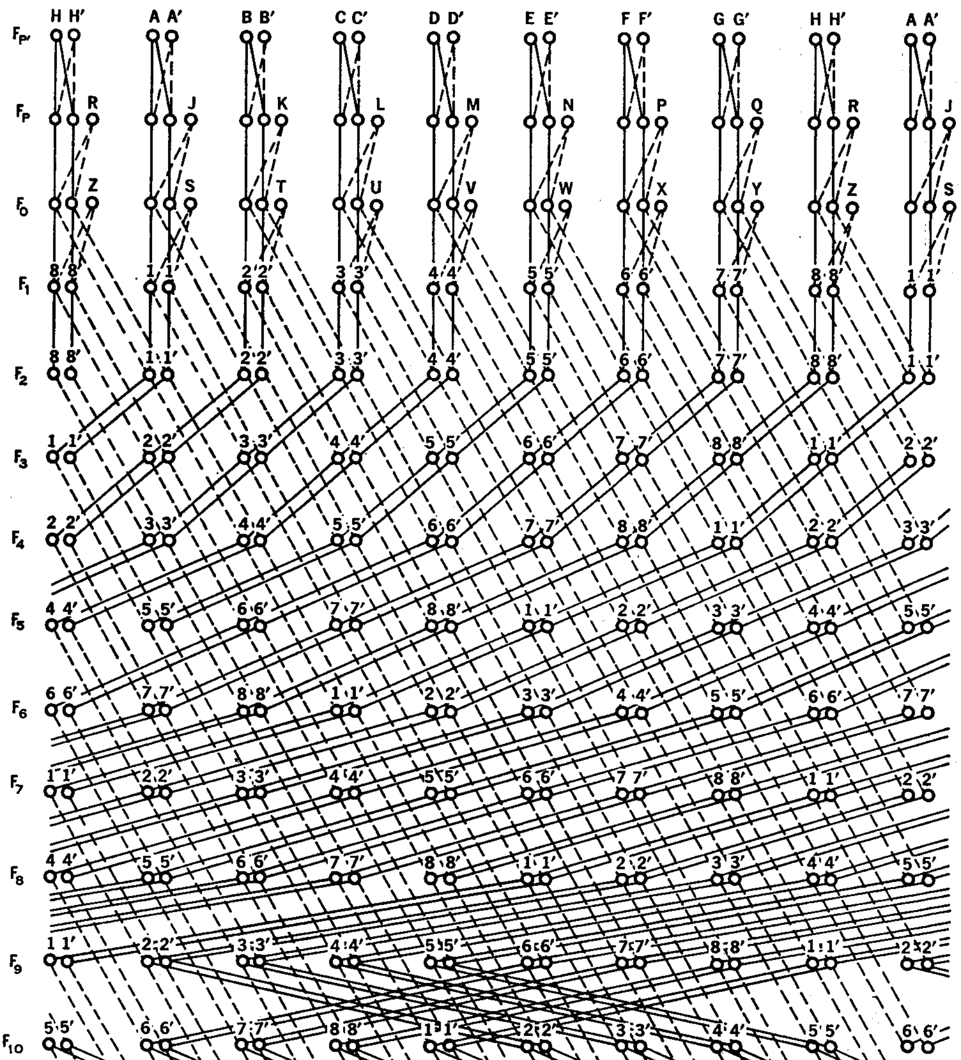

FIG. 2. Alternative mating system to establish a breed of honeybees from 24 lines.

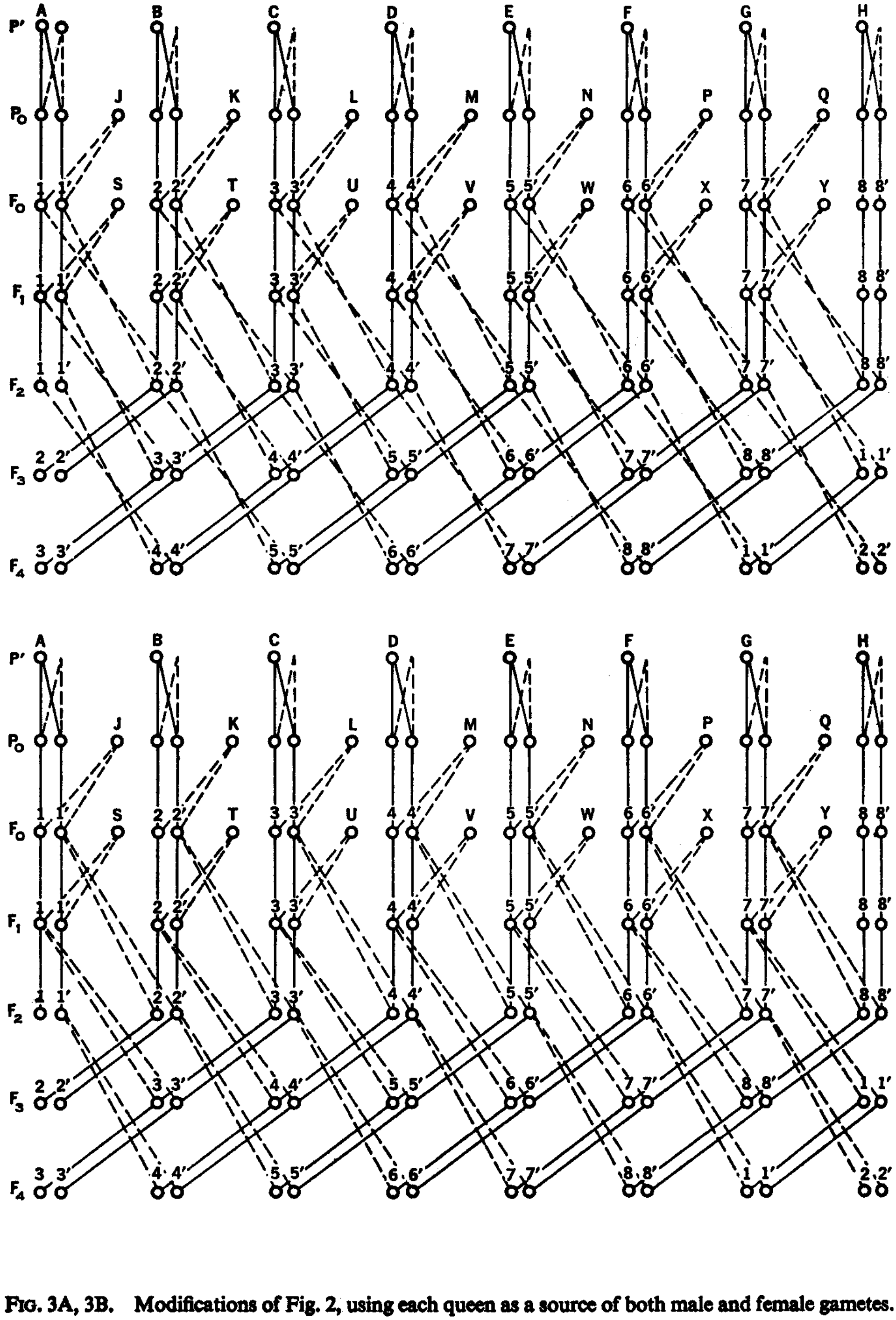

FIG. 3A, 3B. Modifications of Fig. 2, using each queen as a source of both male and female gametes.

queens in each generation, instead of one line using 16 queens in each generation. Obviously, the loss of heterozygosity is greater in the second plan. However, in the alternative plans shown in Fig. 3A and 3B, the 16 queens comprise one mating population. We anticipate that, when we reach the F_5 generation, we shall adopt one of the latter breeding systems to develop our selected breed. In the earlier generations it seems advisable to keep the two parallel lines separated to maintain variability, so that selection can be more effective.

In establishing this Italian standard breed, selection is practised in each generation in an effort to produce and maintain an improved breed. Once this breed is established and is in demand by commercial interests, instrumentally inseminated breeding queens can be obtained from the Stock Center. Their daughters will then serve as the source of drones for mating with queens of the next generation, as indicated in Table 1. If the desired line crosses are to be obtained, and the probabilities of pure natural matings are to increase in each generation, the selection of breeding queens outlined in Table 1 must be rigorously followed.

TABLE 1. Circular mating plan for honeybees. Mate queens of F generations to drones from queens shown in squares. *Example:* In 1971 queen 5_4 was mated to drones from queen 1_3, $6_4 \times 2_3$, etc.

	Queen sub-line							
Generation	1	2	3	4	5	6	7	8
F_1	8_0	1_0	2_0	3_0	4_0	5_0	6_0	7_0
F_2	7_1	8_1	1_1	2_1	3_1	4_1	5_1	6_1
F_3	6_2	7_2	8_2	1_2	2_2	3_2	4_2	5_2
F_4	5_3	6_3	7_3	8_3	1_3	2_3	3_3	4_3
F_5	4_4	5_4	6_4	7_4	8_4	1_4	2_4	3_4
F_6	3_5	4_5	5_5	6_5	7_5	8_5	1_5	2_5
F_7	2_6	3_6	4_6	5_6	6_6	7_6	8_6	1_6
F_8	8_7	1_7	2_7	3_7	4_7	5_7	6_7	7_7
F_9	7_8	8_8	1_8	2_8	3_8	4_8	5_8	6_8
F_{10}	6_9	7_9	8_9	1_9	2_9	3_9	4_9	5_9
F_{11}	5_{10}	6_{10}	7_{10}	8_{10}	1_{10}	2_{10}	3_{10}	4_{10}
F_{12}	4_{11}	5_{11}	6_{11}	7_{11}	8_{11}	1_{11}	2_{11}	3_{11}
F_{13}	3_{12}	4_{12}	5_{12}	6_{12}	7_{12}	8_{12}	1_{12}	2_{12}
F_{14}	2_{13}	3_{13}	4_{13}	5_{13}	6_{13}	7_{13}	8_{13}	1_{13}
F_{15}	8_{14}	1_{14}	2_{14}	3_{14}	4_{14}	5_{14}	6_{14}	7_{14}
F_{16}	7_{15}	8_{15}	1_{15}	2_{15}	3_{15}	4_{15}	5_{15}	6_{15}
F_{17}	6_{16}	7_{16}	8_{16}	1_{16}	2_{16}	3_{16}	4_{16}	5_{16}
F_{18}	5_{17}	6_{17}	7_{17}	8_{17}	1_{17}	2_{17}	3_{17}	4_{17}
F_{19}	4_{18}	5_{18}	6_{18}	7_{18}	8_{18}	1_{18}	2_{18}	3_{18}
F_{20}	3_{19}	4_{19}	5_{19}	6_{19}	7_{19}	8_{19}	1_{19}	2_{19}

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