# VIABILITY AND SEX DETERMINATION IN THE HONEY BEE (APIS MELLIFERA L.)

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A FTER WHITING (1940a, 1943) established conclusively that sex in the parasitic hymenopteron Habrobracon is controlled by multiple sex alleles, it became of interest to determine whether the same mechanism exists in other insects, in which males develop from unfertilized eggs and are haploid. The work described in this paper was started in 1944 at the SOUTHERN STATES BEE CULTURE LABORATORY, of the BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, to determine whether sex is controlled by the same mechanism in the honey bee. In this insect the problem was also of practical interest because it involved egg hatchability, which is of vital importance in the building up of colony populations for honey production.

According to WHITING, sex in *Bracon hebetor* Say [2] is controlled by a series of sex alleles (*xa, xb, xc,* etc.). Females are heterozygous (*xa/xb, xa/xc,* etc.), and when unmated produce haploid sons of two types in equal numbers (*xa* and *xb, xa* and *xc,* etc.). When females are mated, about two-thirds of their eggs are fertilized. If the parents have different sex alleles (*xa/xb × xc,* for example), all fertilized eggs develop into females (*xa/xc* and *xb/xc*). This is called a three-allele fraternity. If one of the sex alleles of the female parent is identical with that of the male parent (*xa/xb × xa* for example), then one-half the fertilized eggs are heterozygous (*xa/xb*) and develop into females, and one-half are homozygous (*xa/xa*) and highly inviable but sometimes develop into adult biparental males. This is called a two-allele fraternity. Haploid males are highly viable. Inbreeding tends to bring together identical sex alleles, resulting in low viability, whereas in outcrosses such combinations are less likely and good viability usually occurs. WHITING has already demonstrated nine alleles, and there are probably many more present in the wild population. Their allelic nature has been demonstrated by linkage tests.

Although *Bracon hebetor* is the only species in which the existence of multiple sex alleles has been proved, genetic work suggests that the sex-determining mechanism is the same in other members of the genus and in other Hymenoptera in which outcrossing is common. In *B. brevicornis* Wesmael, SPEICHER and SPEICHER (1940) found biparental males. INABA (1939) has

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[1] In cooperation with LOUISIANA STATE UNIVERSITY.
[2] Usually treated in WHITING's papers under the name *Habrobracon juglandis* (Ashm.). On account of its familiarity to geneticists the term "*Habrobracon*" is retained in this paper.

reported biparental males and evidence for multiple sex allelism in *Habrobracon pectinophorae* Watanabe, but this species seems to be identical with *Bracon hebetor* (WHITING 1949). In the chalcidoid wasp *Pteromalus puparum* (Linnaeus) genetic evidence obtained by DOZORCEVA (reviewed by WHITING 1940b) suggests two- and three-allele fraternities and multiple alleles. In these forms, in which outcrossing is the rule, the waste of reproductive effort through low viability would not be a serious matter. In close-crossed species, however, where frequent two-allele fraternities would cause a great waste of eggs, the multiple-allele scheme does not seem to apply in the species that have been studied. In Melittobia, in which mother-son and sibling matings are the rule with rare outcrossing, SCHMIEDER and WHITING (1947) found neither diploid males nor low viability in close crosses—an indication that the method of sex determination may be different. In another naturally close-crossed species, *Telenomus fariai* Lima, DREYFUS and BREUER (1944) report no biparental males and present cytological data to show that females are heterozygous for sex, having X and Y chromosomes which are distinguishable. The males developing from unfertilized eggs have an X chromosome in all the somatic cells and in the early spermatogonia. In spermatogenesis the X is reduced to a Y by loss of a fragment, so that all sperms are Y-bearing. It is assumed that through differential maturation in oogenesis the Y passes into the polar body and that the egg pronucleus always receives an X. MANNING (1949) describes a similar mechanism in the honey bee, except that there is no Y chromosome. All eggs become X-bearing through differential maturation, and in spermatogenesis the X is extruded so that all sperms contain only a set of autosomes. Two sets of autosomes plus an X determine femaleness, and one set of autosomes plus an X determines maleness.

Since the honey bee is naturally outcrossed, we should expect its sex mechanism to conform more closely to that of Habrobracon than to that of close-crossed species. This paper describes viability tests and gives relevant data that suggest conformity to the Habrobracon scheme.

## MATERIALS AND METHODS

If in Habrobracon we start with a three-allele mating, such as $xa/xb \times xc$, and make individual sibling matings for two generations, viability will follow a definite pattern. In the first generation one-half of the fraternities will be the two-allele type, exhibiting low viability of zygotes, and one-half will be of the three-allele type, exhibiting high viability of zygotes. In the second generation, if we breed from a low-viability (two-allele) fraternity, only low-viability fraternities will result, and if we breed from a high-viability (three-allele) fraternity, the results will be the same as in the first generation.

The same plan was followed with the honey bee. A naturally mated queen producing progeny of high viability was chosen. Daughter queens were mated individually to their brothers, and the worker progeny (from fertilized eggs in worker cells) tested for viability to the fifth day. Granddaughter queens

were then reared from one of the daughters producing highly viable worker progeny and from one producing poorly viable worker progeny, and mated individually to their brothers. The resulting worker progeny were tested for viability to the fifth day, and some to the nineteenth day just before emergence. The nineteenth-day counts were not made at random, but in all tests conducted after a certain date.

Mating was controlled as completely as is possible in the honey bee. All matings were by artificial insemination by the method described by MACKENSEN and ROBERTS (1948). The insemination syringe was sterilized between matings. All drones were reared in colonies stocked with bees of a distinguishable color, so that drones from laying worker bees could be recognized and discarded.

A viability test was made as follows: When the queen started laying, the nucleus (small colony) was strengthened with bees and brood if this seemed necessary. The queen was caged in an excluder cage (one which permits the passage of bees but not of the queen) on a specially prepared or selected comb. Such a comb was usually taken from another nucleus—a comb in which the queen had not yet started laying but in which the worker bees had already polished the cells in a well-defined area surrounded by pollen. When such combs were not available, a clean comb was prepared by adding pollen in a ring around the central area. The queen was restricted to one side of the comb for 24 hours and then to the other side for a similar period. The excluder cage was then removed and the queen confined in a small screen cage hung in the nucleus to prevent excessive production of queen cells. At the end of each 24-hour laying period the eggs were counted in a rectangular portion of the laid area, and the larvae were also counted on the fifth day. At the last count the oldest larvae were therefore five days old and the youngest four days old. Eggs normally hatch in 76 hours, but it is so difficult to see them that they cannot be counted in great numbers.

The viability tests on the second generation were made without the use of an excluder cage. A nucleus hive containing three standard Langstroth frames was used. As soon as the queen was laying well, the center comb was simply replaced with a selected or prepared test comb. Providing side combs solidly filled with honey and pollen forced the queen to lay in the test comb. After the queen had layed for 24 hours, the eggs were counted. Counts were again made on the fifth and nineteenth days.

These counts do not record the time of death, but the number of individuals remaining apparently alive at the various stages. Although nurse bees remove dead remains quickly, there is certain to be some delay. In these tests shriveled eggs and dead larvae or pupae were rarely seen, and a check of sealed brood showed that a count of the normally capped cells on the nineteenth day was accurate.

Viability tests were also made on drone eggs (unfertilized). Since these tests were made during the ·summer, when queens are unlikely to lay drone eggs and bees are unlikely to care for them properly, special methods were

necessary. Special combs were prepared by inserting rectangular pieces of drone comb about 3 by 4 inches in the center of a worker comb. Pollen was packed into the cells at both ends of each comb, so that only a small area of empty worker cells surrounded the drone cells and the queen was thus forced to lay in them. In other respects the method was similar to that described for worker brood. Often several days elapsed before eggs were laid in the drone comb.

Mated queens ordinarily lay only fertilized eggs in worker cells and un-fertilized eggs in the larger drone cells. However, artificially mated queens sometimes receive so few sperms that some of the eggs laid in worker cells are not fertilized. Such queens are called partial or complete drone layers, as the case may be, and can be recognized by the appearance of the cappings of sealed brood, the cappings over drone larvae in worker cells being more rounded than those over worker larvae. Eleven such queens were excluded from the worker brood viability data.

A queen forced to lay on drone comb will often lay fertilized eggs. As in worker comb, worker larvae are capped flat and drone larvae rounded. Rarely was a flat capping found in the drone brood tested in these experiments.

<div align="center">RESULTS</div>

<div align="center">*Viability tests on worker brood*</div>

Of the eight queens tested in the first generation, four produced brood of low viability to the fifth day—45, 45, 48, and 49 percent; the other four produced brood of high viability—90, 95, 96, and 97 percent. At least 742 eggs of each queen were counted.

The second-generation queens are divided into two groups: (1) daughters of a first-generation queen producing brood of low viability—45 percent on the fifth day; and (2) daughters of a first-generation queen producing brood of high viability—90 percent on the fifth day. The results are given in table 1. In the first group all 34 progenies tested were poorly viable, ranging from 43 to 66 percent, with an average of 51 percent. Twenty-seven tests carried to the nineteenth day revealed brood ranging from 32 to 49 percent in viability, with an average of 43 percent. Progenies of the second group fell definitely into a high and a low class, as in the first generation, with 10 in the low class and 16 in the high class. On the fifth day the low class ranged from 41 to 59 percent (average 52), and the high class from 88 to 97 percent (average 94). On the nineteenth day the corresponding figures for tests carried to this stage were 34 to 46 percent (average 43), and 85 to 97 percent (average 91).

The nineteenth-day counts showed that total viability remained in the high class when the fifth-day count was in the high class, and that the nineteenth-day viability was definitely low in tests in which the fifth day count was in the upper part of the low-viability class. By this time the two highest fifth-day counts (66 percent each) were reduced to 34 and 48 percent.

To determine at what age death usually occurs, more frequent counts were made on brood of two queens producing low-viability brood. The results are

TABLE 1

*Viability of worker brood produced by second-generation queens.*

| Brood of daughters of a first-generation queen producing poorly viable brood | | | Brood of daughters of a first-generation queen producing highly viable brood | | |
|---|---|---|---|---|---|
| Number of eggs | Percentage surviving | | Number of eggs | Percentage surviving | |
| | 5th day | 19th day | | 5th day | 19th day |
| 125 | 66 | 34 | 528 | 53 | .... |
| 714 | 55 | .... | 484 | 54 | 46 |
| 577 | 50 | 49 | 621 | 52 | 34 |
| 980 | 48 | 46 | 674 | 51 | 45 |
| 854 | 54 | 41 | 819 | 54 | .... |
| 696 | 49 | 39 | 726 | 57 | 45 |
| 501 | 50 | 43 | 849 | 53 | .... |
| 936 | 49 | 46 | 810 | 41 | 39 |
| 279 | 55 | 32 | 903 | 49 | 46 |
| 593 | 66 | 48 | 943 | 59 | .... |
| 371 | 49 | 47 | .... | .... | .... |
| 911 | 50 | .... | .... | .... | .... |
| 566 | 54 | 35 | 975 | 96 | 93 |
| 328 | 52 | .... | 370 | 95 | 88 |
| 484 | 50 | .... | 765 | 93 | 90 |
| 804 | 46 | 45 | 921 | 97 | 97 |
| 774 | 49 | 47 | 696 | 96 | .... |
| 600 | 48 | 45 | 857 | 97 | 96 |
| 825 | 51 | 48 | 649 | 93 | 91 |
| 446 | 50 | .... | 884 | 94 | 89 |
| 562 | 43 | 40 | 518 | 88 | 86 |
| 688 | 48 | 45 | 863 | 97 | 94 |
| 415 | 47 | 43 | 514 | 93 | 92 |
| 418 | 50 | 47 | 763 | 93 | .... |
| 259 | 45 | .... | 859 | 94 | .... |
| 216 | 49 | 40 | 319 | 91 | 87 |
| 720 | 53 | 44 | 428 | 97 | 96 |
| 725 | 49 | 47 | 344 | 95 | 85 |
| 602 | 49 | 44 | .... | .... | .... |
| 851 | 48 | .... | .... | .... | .... |
| 487 | 61 | 42 | .... | .... | .... |
| 649 | 48 | 47 | .... | .... | .... |
| 900 | 50 | 49 | .... | .... | .... |
| 660 | 57 | 36 | .... | .... | .... |

given in table 2. The greatest loss occurred between the third- and fourth-day counts or just at the time of hatching, as in Habrobracon. The viability did not drop below 50 percent until after the ninth day. Apparently some of the homozygotes live to this stage.

## Viability of drone brood

Tests were made on drone brood to determine the viability of unfertilized eggs. Two tests on the drone brood of a queen whose worker brood was 45 percent viable on the fifth day gave the following results: 232 eggs tested 94 percent viable, and 261 eggs tested 83 percent viable. Three tests on drone brood of another queen, whose worker brood tested 48 percent viable on the

TABLE 2

*Percentage survival of low-viability broods from two queens.*

| Queen No. | Number of eggs | Days after beginning of test | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | 7 | 9 | 11 | 13 | 19 |
| S-8 | 484 | 98 | 95 | 60 | 54 | 53 | 52 | 47 | 46 | 46 |
| S-17 | 726 | 97 | 94 | 59 | 57 | 56 | 52 | 47 | 46 | 45 |

fifth day, gave the following results: 188 eggs tested 65 percent viable; 164 eggs, 90 percent viable; and 229 eggs, 74 percent viable. In low-viability fraternities, therefore, unfertilized eggs are more viable to the fifth day than fertilized eggs.

Results are also available from tests on the brood of six mated queens that produced nearly 100 percent of drone brood. The intention being to test fertilized eggs before it was known that the queens were drone layers, the tests were conducted in worker combs. The number of eggs tested and the per cent viable to the fifth day in these tests were as follows: 219, 87 percent; 244, 83 percent; 341, 89 percent; 167, 91 percent; 369, 88 percent; and 230, 90 percent. Later counts were not attempted because many drones die from lack of room in worker cells. Data from all these drone-brood tests show that viability is associated with fertilization, as in Habrobracon.

### Tests for biparental males

In Habrobracon biparental males can occur when identical sex alleles come together in a mating. They are phenotypically recognizable when a recessive female is mated to a wild-type male. In such a mating uniparental haploid male progeny are recessive and the biparental diploid males are wild-type. In the honey bee a further requisite for the absolute identification of biparental males is that they be reared in a colony stocked with bees of a contrasting body color, so that the haploid sons of wild-type laying workers, which occasionally occur in any normal hive, can be distinguished from the diploid biparental sons of the queen.

The first attempt to recognize biparental males was made in a line carrying the recessive mutant white, causing a complete lack of pigment in the eyes. This line was maintained by sibling matings in such a way that biparental males could be recognized every other generation. No such males were found, although for several generations thousands of biparental individuals emerging from worker comb were examined for each generation. In this material conditions were not the most favorable, because it was not certain that the line had been reduced to two alleles.

Another attempt was made in a stock carrying the recessive mutant cordovan, which changes the black body pigment to various shades of brown, cordovan appearing in the thorax. This stock was supplied by the DIVISION OF BEE CULTURE, Beltsville, Maryland. Heterozygous queens were mated individually to their brothers, and a two-allele mating, one producing brood

of low viability, was then chosen to continue the line. Homozygous cordovan daughters were then mated to wild-type brothers. Viability of the worker progeny of these matings was low, the line having apparently been reduced to two alleles as expected. Biparental males were expected to be of the wild type in contrast to the cordovan of the usual haploid males. The progeny of two queens were examined. The queens were caged on a fresh comb every few days. Drone comb was used, so that all abnormal individuals, biparental males, haplo-diploid males, or sex mosaics had the advantage of developing in large cells. Of the 12,533 individuals examined, 8,603 were wild-type workers, 3,929 were cordovan drones, and 1 was a sex mosaic with the female parts wild-type and male parts cordovan.

<div align="center">DISCUSSION</div>

The parallelism between Habrobracon and the honey bee brought out in these experiments strongly suggests that the mode of sex determination is the same in the two insects. At least there seems to be in the honey bee a series of haploviable homozygous lethal alleles similar in lethal action to the sex alleles of Habrobracon. That such a lethal mechanism is at work, and that it is probably universal in the honey bee, lending support to its association with sex, is amply substantiated by experience in bee breeding since these experiments were made. Mother-son (two-allele) matings in material from various sources representing two races have invariably resulted in low viability. Two-allele lines established by such matings have continued to show low viability and to breed as two-allele lines. Crosses by individual mating between two-allele lines fall into one of three classes as expected: (1) all progenies of low viability, showing that the alleles are the same; (2) all progenies highly viable, showing the alleles to be different; and (3) one-half the progenies highly viable and one-half poorly viable, showing that the two lines have one allele in common. This procedure is now being used to establish two-allele tester lines with definitely identified alleles for use in determining the alleles of any unknown breeding stock.

Although biparental males, which are necessary to prove a relation to sex, were not found, apparently many of the homozygotes live to various stages of larval life. In low-viability broods the survival counts on the fifth day average higher than 50 percent, and in table 2 the ninth day counts are higher than 50 percent. Perhaps by selection a strain can be developed in which some live to maturity.

If sex is determined as MANNING suggests, then the lethal alleles must be autosomal and have no connection with sex. It is difficult however to imagine the survival of such a wasteful series of lethals unless it is associated with something as vital as sex.

Since inbreeding often brings about low viability and a waste of eggs and young larvae, we would expect the honey bee to have developed behavior patterns that promote outbreeding. This is the case. Mating does not take place in the hive where severe inbreeding would result, but on the wing where

drones from other colonies are available. In the wild state bees live in hollow trees and cliffs and are widely scattered. A queen mating near her own hive would probably meet a drone from her own colony—one of her brothers. Mating at a distance would increase the chance of meeting a drone from another colony. Some observations indicate that virgin queens do mate at some distance from their own colony.

In a favorable open location fifty virgin queens were followed with the eye until they either met drones or passed beyond the range of vision. After leaving the hive the virgins flew in ever-increasing circles until a radius of about 50 feet was reached, and then headed straight away from the queen yard. Two of the virgins were caught by drones while still circling and they dropped toward the ground, but neither drone nor queen could be found on the ground. The others were followed closely by one or more drones as they circled, but as they headed away from the queen yard they invariably were alone until they disappeared from view. From these observations it appears that they deliberately avoid drones near their own hive.

## PRACTICAL CONSIDERATIONS

One of the main factors in the honey production of the bee colony is its population, which in turn is largely dependent upon the laying capacity of the queen and the efficiency within the brood nest. The number of eggs a queen can lay in a day is limited, and if half her eggs and young larvae die, as in a two-allele mating, the queen does not have the capacity to lay the additional eggs required to maintain the colony at full strength. In addition, the efficiency within the brood nest is reduced when half the eggs and young larvae occupy cells for three days or longer only to die and be removed. The brood becomes spotted and the queen cannot follow her regular laying pattern, but must hunt for scattered empty cells. This inefficiency may be a very critical factor early in the spring, when the brood nest is limited to a small space that the bees can keep warm.

The fact that about one-half of all queens mate a second time (ROBERTS 1944) reduces the amount of very low viability, because under the usual conditions existing in queen yards a queen is not likely to meet a drone with a similar lethal allele at both matings. Theoretically, the brood of twice-mated queens ranges in viability from 50 to 100 percent depending on the types and proportion of types of sperm received. Although minimized by double mating, the effect of lethal alleles is still serious, especially because the reduction in efficiency is often too slight to be readily recognized by the beekeeper.

It is therefore greatly to the beekeeper's advantage to have his queens properly mated as to lethal alleles. He must avoid inbreeding and select for good brood quality to keep as many alleles in the strain as possible. The most convenient way for the queen producer to do this is to be sure that, for each generation, the breeder queens (already mated) he uses as a source of virgins are descended from different mothers than those he uses as a source of drones. Each season he should use several breeder queens for the production of vir-

gins and several for the production of drones. When too much brood of poor quality appears, he can outcross to an unrelated strain. A system of criss-crossing strains might be advantageous. Finally, with the aid of artificial insemination, he can establish and maintain inbred lines containing known sex alleles and then cross them in the best combination. The last method is beyond the facilities of the individual breeder, but can be carried out by cooperative and agricultural research agencies which can distribute foundation stock for the production of hybrids by the commercial queen producer.

## SUMMARY

Experiments were conducted at the SOUTHERN STATES BEE CULTURE LABORATORY, of the BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, to ascertain whether the sex-determination mechanism in the honey bee (*Apis mellifera* L.) is similar to that of the parasitic hymenopteron Habrobracon, in which there exists a series of sex-determining alleles, $xa$, $xb$, $xc$, etc. Heterozygotes are females, azygotes males, and homozygotes biparental males which are highly inviable.

Evidence was presented which supports the existence of a similar mechanism in the honey bee. When individual sibling matings were made for two generations, the viability of the worker progenies followed the same pattern expected if similar matings were made in Habrobracon. Although the data obtained show the existence of a series of haploviable homozygous lethal alleles similar in their lethal action to the sex alleles of Habrobracon, a relation to sex was not established because biparental males were not found in crosses of homozygous recessive females to wild-type males.

In sex determination the outcrossed honey bee is apparently more like Habrobracon and other outcrossed Hymenoptera than like the close-crossed species in which neither biparental males nor low viability occur in close crosses.

Observations are recorded which indicate that queens have a natural tendency to mate away from their own hives, thus avoiding inbreeding. Since inbreeding brings together similar lethal alleles and lowers brood viability, colony population, and honey production, it should be carefully avoided in commercial beekeeping practice.

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