BEE GENETICS[1,2]

WALTER C. ROTHENBUHLER, JOVAN M. KULINČEVIĆ
The Ohio State University, Columbus, Ohio, U.S.A.

WARWICK E. KERR
Universidade de São Paulo, Ribeirão Prêto, Brazil

INTRODUCTION

Certain characteristics of honey bees attract and others repel investigators. Among the former is the haplodiploid origin of the two sexes. The haploid males (drones), having arisen from unfertilized eggs, constitute a case in which an animal developed from a gamete may be studied. In spermatogenesis each drone multiplies its own genotype a few million times. The two kinds of diploid females (queens and workers), although genetically identical, are highly dimorphic as a result of environmental differences in rearing, and display very different behaviors and physiologies. A queen lives ordinarily two or three years and can produce hundreds of thousands of male and female offspring. Workers live a much shorter time, but are rich in highly evolved behaviors. They function in the integrated colony as nest builders and defenders, food gatherers, brood nurses, brood nest cleaners, and air conditioners in both summer and winter. Of genetical interest also are the great differences in behavior among races and strains of bees.

But there is another side. Bees are so admirably equipped to defend their nest that they frighten away much interest. If their rearing and management were less complex, they would be favored in many laboratories. If their mating habits were not so cavalier, there might be a paper today on bee genetics authored by Gregor Mendel. He kept bees, experimented with them, and tried unsuccessfully to control their mating by use of cages (43, 91). The problem of controlling mating has now been solved and the honey bee is one of the few insects (perhaps the only one) in which artificial insemination is a completely reliable procedure (58, 78, 143).

Classification and geographical variation.—The common honey bee, *Apis melifera L.,* is one of five accepted species in the genus *Apis*. Except for *A. mellifera,* the best known one is *Apis cerana* Fabr. Three other species, *Apis dorsata* Fabr. (giant honey bee), *Apis florea* Fabr. (little honey bee), and *Apis andreniformis* Smith, are residents of southern and southeastern Asia. They are known mainly by scientists, and do not have a special role in beekeeping and bee genetics. *A. andreniformis* is least known, but accord-

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ing to Michener (82) is a distinct species looking somewhat like *A. florea,* especially in its small size. The relationships of the genus *Apis* to other systematic units of Hymenoptera were presented graphically in a recent review (108).

Geographical variations of *A. mellifera* and *A. cerana* have been classified in many different ways by Buttel-Reepen (13) Ruttner & Mackensen (116), Maa (72), Kerr & Laidlaw (54), and Goetze (30). Recently Dupraw (22, 23, 24, 25) introduced non-Linnean taxonomy into the systematics of honey bees. It seems that this is a very significant improvement in the field because it is possible to recognize the native geographical origin of honey bees by minimal material (24). After comparison of Linnean and non-Linnean methods it became clear that *A. mellifera* and *A. cerana* are evolutionarily two different species of honey bees. *A. mellifera* includes the European-African complex of bees and those of the western part of Asia. *A. cerana* is found in southern and eastern Asia, on many Indonesian islands, and in Japan. This distribution is shown in Figure 1.

Our division of *A. mellifera* into four groups is being done after consideration of Goetze's suggestions and Dupraw's results of non-Linnean taxonomy and systematics of honey bees. In these groups are included and classified all known local variants often based on small differences in behavioral or economical characteristics. We have no intention of designating the individual variants or the groups as subspecies, but are instead providing a key to the terminology the reader will encounter.

Into the African group we put all bees south of the Sahara and *lamarckii* from the Egyptian region. *A. m. lamarckii* is included in the African group by Dupraw (24), but Geotze (30) had *lamarckii* in the Middle Eastern group.

The Middle Eastern group makes a transition between the African and the Southeast European groups (24, 30). The Southeast European group includes *carnica, cecropia, ligustica* and all the bees of Southeastern Europe which are related to the Middle Eastern group but developed independently (28, 113) of the Northwestern European group. The division was caused by the Alps mountain chain in glacial times.

The Northwest European group is related to African bees through North African *intermissa* bees. *A. m sahariensis,* a yellow form of North African bee, tends to be close to the African group (24). Goetze (30) and Ruttner (113) suggested that the divergence between Southwestern and Northwestern European bees arose when honey bees colonized Europe from Africa and the Middle East. Bährmann (7) reported comparative morphological studies of 16 body characteristics from the Northwest European group, and in spite of the vast distribution, it was very uniform. From all these so-called geographical races, *mellifera, ligustica, carnica,* and *caucasica* are more widely accepted in beekeeping.

In *A. cerana* we cannot find so clear a differentiation as in *A. mellifera.*

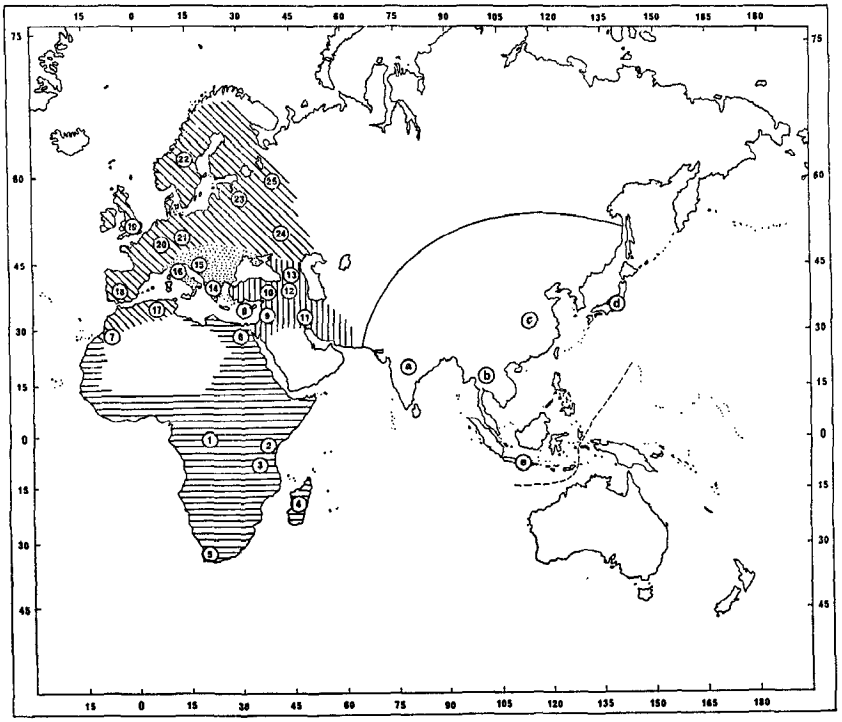

FIG. 1. Geographical variants of *Apis mellifera L.* and *Apis cerana* Fabr.

A. mellifera L.

I African group
- 1. *adansonii*
- 2. *monticola*
- 3. *litorea*
- 4. *unicolor*
- 5. *capensis*
- 6. *lamarckii*

II Middle Eastern group
- 8. *cypria*
- 9. *syriaca*
- 10. *anatolica*
- 11. *persica*
- 12. *armenica*
- 13. *caucasica*

III Southeast European group
- 14. *cecropia*
- 15. *carnica*
- 16. *ligustica*

IV Northwest European group
- 7. *sahariensis*[a]
- 17. *intermissa* (tellica)
- 18. *iberica*
- 19. *domestica*
- 20. *mellifera*
- 21. *nigra*
- 22. *lehceni*
- 23. *silvarum*
- 24. *acervorum*
- 25. *uralica*

A. cerana Fabr.
- a. *indica*
- b. *socialis*
- c. *sinensis*
- d. *cerana* (japonica)
- e. *javana*

[a] *A. m. sahariensis* is closely related to the African group (Dupraw, 1965).

Goetze (30) recognized five geographical races of *A. cerana* (Fig. 1), but Dupraw (24) by non-Linnean analysis recognized only *A. cerana indica* and *A. cerana cerana,* sometimes known as *A. c. japonica.* It is concluded that the bees of Asia do indeed represent a single population which Dupraw (24) separates from another more complex population in Europe and Africa.

Hachinohe (32) attempted to inseminate *A. mellifera* females with sperms from *A. cerana japonica.* Semen was difficult to collect, but some female progeny were obtained. The experiments had to be terminated before queens were reared.

Cytology and Cytogenetics

All recent studies are in agreement that gonial and early blastoderm cells of *Apis mellifera* show 16 chromosomes for males and 32 for females (33, 117). High degrees of polyploidy, however, are found in differentiated somatic tissues (80, 96, 117, 118).

Chromosome numbers in the Indian species of honey bees have been investigated recently. *A. cerana,* like *A. mellifera,* shows 16 in the male and 32 in the female (17, 18, 120). It is a very interesting surprise to learn that *A. florea* and *A. dorsata* show eight chromosomes in males and 16 in females (17, 132). These facts support the suggestion by Kerr (47) that polyploidy has played an important role in the evolution of bees.

The maintenance of chromosome number in gametogenesis of haploids requires some modification of meiosis. The traditional explanation has involved the production of a nonnucleated cytoplasmic bud as one of the two products of an abortive meiosis I followed by an equational division of chromosomes in meiosis II, but this interpretation has been challenged (142). The detailed, careful study by Sharma, Gupta & Kumbkarni (120) on spermatogenesis in *A. cerana* gives clear evidence of the formation of a cytoplasmic bud in meiosis I and also shows that a spherical, proteinaceous, cytoplasmic body moves into the cytoplasmic bud and is probably discarded with it.

Some other cytologically unusual phenomena are found in bees. Unfertilized eggs of most races of bees usually develop into males. By contrast, worker bees of *A. mellifera capensis* can produce unfertilized eggs most of which develop into females (44, 89, 90). In fact, a colony can be maintained queenless for months by this kind of reproduction, with no male brood seen, and it can eventually requeen itself (4). Queens can be artificially reared using larvae from laying workers (52). Mackensen (73) showed that the production of females from unfertilized eggs occurred in frequencies of 1 or 2 per cent in three genetically different lines of *A. mellifera* in America. It is suspected that production of females by laying workers occurs generally in all common honey bees (52). Only the high frequency of female production from unfertilized eggs, and the high frequency of laying workers in queenless colonies of the Cape bee distinguishes it.

Tucker (136) investigated the mechanism underlying production of fe-

males from unfertilized eggs. He found their production highest when the queen's oviposition rate was accelerated. Kerr & Araujo (52) independently found the same thing in *capensis* virgin queens. Based on the genotype of female progeny from unmated genetically marked heterozygous queens, Tucker suggested that a misorientation of the first meiotic spindle occurs, and two pronuclei are produced by meiosis II instead of one pronucleus and three polar nuclei. The two then unite to form a zygote.

F_1 worker bees from a *capensis-ligustica* cross do not have the ability to produce large numbers of females from unfertilized eggs (51).

Genetic evidence has established that gynandromorphic honey bees usually originate from a zygote and one or more accessory sperms. The zygote resulting from normal syngamy of a single egg with a single sperm pronucleus develops into female tissue, whereas the accessory sperm or sperms give rise to male tissue (103, 109). The tendency of the egg to permit or facilitate development of accessory sperms was increased by selection from far less than 1 per cent of eggs to 40 or 50 per cent (101). Cold shocks of newly laid eggs have been shown to increase gynandromorph production, and the same mechanism as above, polyspermy and androgenesis, is involved (20). More recently, Drescher (19) has been able to raise gynandromorph production to 32.5 per cent and lower it to 6 per cent in the progeny of one queen by inducing an increase or a decrease respectively in the queen's oviposition rate.

Genetically mosaic females have been found which probably arose from doubly fertilized binucleate eggs (129).

Completing the circle of anomalies, Laidlaw & Tucker (64) by genetic evidence established that two accessory sperms can unite in the cytoplasm of the egg and give rise to diploid female tissue.

PHENOGENETICS

Major genes: their allelic and linkage relationships.—Table 1 lists the major genetic mutants known for *A. mellifera.* The first mutation in the list is known only in *A. mellifera adansonii.* Several of these mutants no longer exist, *Droopy, Schwarzsüchtig, Rudimental wing, lethal,* and the ***white eye*** of Michailoff being among them. Stocks of many of the others are maintained by Dr. Harry H. Laidlaw with the support of the National Science Foundation at the University of California, Davis, California. Mutants may be obtained from Laidlaw's laboratory.

About one-fourth of the possible 2-point linkage tests have been made (Figure 2), and only two cases of linkage between visible mutants discovered. The *hairless* and *chartreuse* loci are linked with about 4.1 per cent crossing over (76), and the *pearl* and *cream* loci are linked with 0.33 per cent crossing over (61).

Lethals have been suspected in several cases. In one case a recessive lethal was found linked with *Rudimental wing.* Crossing over occurred to the extent of 31 per cent (34).

TABLE 1

LIST OF MUTATIONS

Symbol	Name of Mutation	Effect on	When named and by whom		Citations
ac	*brown*	abdominal color	1968	Kerr	51
bk	*brick*	eye color	1953	Laidlaw, Green & Kerr	54, 60, 61, 62, 63, 65, 66, 76
bl	*black*	body color	1962	Laidlaw & el-Banby	59, 61, 62, 139
c	*cordovan*	body color	1951	Mackensen	15, 61, 63, 74, 76, 128a
ch	*chartreuse*	eye color	1952	Rothenbuhler, Gowen & Park	76, 109, 110, 110a
ch^1	*chartreuse-1*	eye color	1953	Laidlaw, Green & Kerr	54, 61, 62, 63, 66
ch^2	*chartreuse-2*	eye color	1953	Laidlaw, Green & Kerr	54, 60, 61, 63, 136
ch^B	*Benson green*	eye color	1964	Laidlaw, el-Banby & Tucker	60
ch^c	*cherry*	eye color	1964	Laidlaw, el-Banby & Tucker	60, 61, 62
ch^r	*red*	eye color	1953	Laidlaw, Green & Kerr	54, 60, 63
cr	*cream*	eye color	1952	Rothenbuhler, Gowen & Park	60, 61, 66, 76, 109, 110, 110a
D	*Droopy*	wings	1952	Rothenbuhler, Gowen & Park	76, 109a, 110a
e	*eyeless*	eye morphology	1965	Laidlaw & Tucker	65
g	*garnet*	eye color	1964	Laidlaw, el-Banby & Tucker	60, 65, 66
h	*hairless*	body hair	1957	Mackensen	76
i	*ivory*	eye color	1949	Rothenbuhler, Polhemus, Gowen & Park	31, 60, 61, 62, 63, 66, 76, 109a, 110, 110a, 110b, 136

TABLE 1 (*Continued*)

Symbol	Name of Mutation	Effect on	When named and by whom		Citations
i^u	*umber*	eye color	1965	Laidlaw & Tucker	66
l	*lethal*	viability	1953	Hachinohe & Onishi	34
m	*modifier*	chartreuse-1 eye color	1953	Laidlaw, Green & Kerr	54, 60, 61, 62, 63, 66
p	*pink*	eye color	1963	Cale, Gowen & Carlile	15
pe	*pearl*	eye color	1964	Laidlaw, el-Banby & Tucker	60, 61, 66
r	*removing*	behavior	1964	Rothenbuhler	107
Rw	*Rudimental wing*	wings	1953	Hachinohe & Onishi	34
S	*Schwarzsüchtig* (*black hairless*)	body hair	1940	Dreher	18a
sh	*short*	wings	1956	Kerr & Laidlaw	54, 61, 62
s	*snow*	eye color	1952	Rothenbuhler, Gowen & Park	31, 60, 66, 76, 109a, 110, 110a
s^t	*tan*	eye color	1964	Laidlaw, el-Banby & Tucker	60, 61, 62
tr	*truncate*	wings	1965	Laidlaw el-Banby & Tucker	61, 62
u	*uncapping*	behavior	1964	Rothenbuhler	107
wr	*wrinkled*	wings	1965	Laidlaw, el-Banby & Tucker	61, 62
	white or ivory	eye color	1931	Michailoff	81
X^1-X^n	*sex alleles*	sex determination	1955	Mackensen	62a, 74, 75, 99, 102

Possible linkage of the sex locus (*X*) with certain other loci has been checked and no linkage found (76). By analysing segregation in impaternate worker progeny of heterozygous unmated queens, Tucker (136) has found that *chartreuse* segregates as if it were 28.8 units from its centromere, and *ivory* segregates as if it were 3.6 units from its centromere.

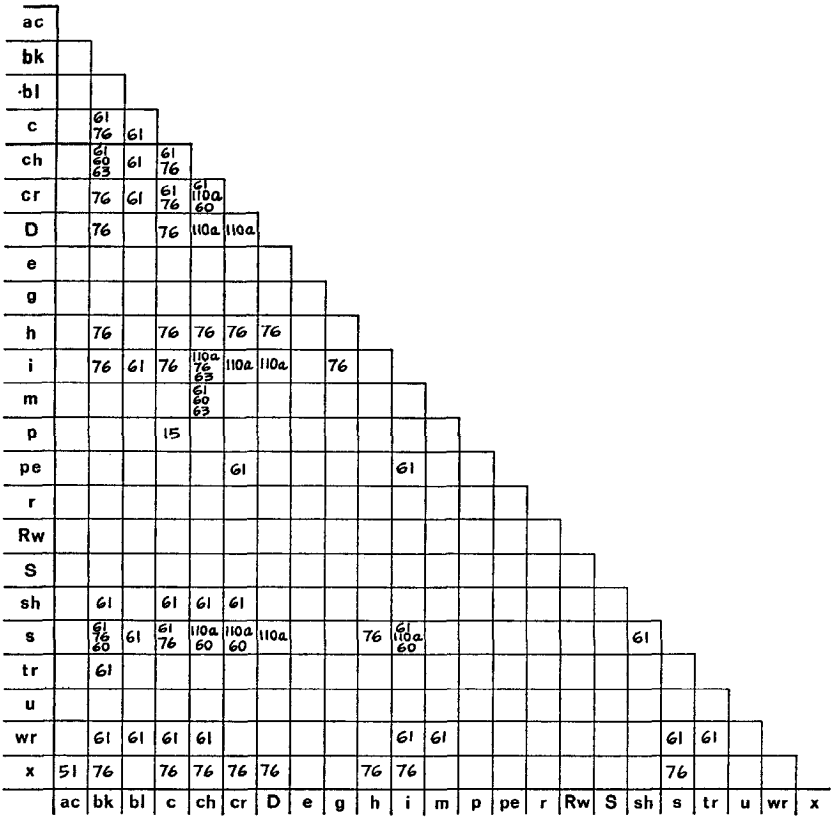

FIG. 2. Diagram of possible linkage studies with citations of those already made.

Sex determination.—In 1925, Bridges wrote, "To me sex-determination in the bee is the outstanding unsolved puzzle, although before the idea of genic balance it seemed one of the clearest and simplest of cases" (12). Haploid males and diploid females are characteristic, not only of bees, but of all the Hymenoptera. The first suggestion of an explanation of sex determination came in the parasitic wasp *Habrobracon juglandis* Ashmead (properly known as *Bracon hebetor* Say) through the work of the Whitings and their collaborators. The *Habrobracon* scheme, (144) in which heterozygotes at a certain locus or chromosome segment (called *X* locus) become females, hemizygotes become males, and homozygotes become males or die, provided a testable hypothesis for the honey bee. Mackensen (74, 75) presented evidence that matings of closely related individuals in which identical sex alleles might be expected in a predictable frequency did indeed suffer a 50 per cent mortality of brood in the predicted proportion of matings. Several investigators looked for diploid males in the progeny of low-

viability matings without success. Eventually, Rothenbuhler (102) found mosaic drones that were combinations of haploid and diploid tissue. Drescher & Rothenbuhler (21) confirmed this finding with more carefully prepared matings. Little doubt of the applicability of the *Habrobracon* mechanism could remain.

Cunha & Kerr (16a) proposed a working hypothesis of sex determination in Hymenoptera designed to generalize Whiting's explanation of sex determination in *Habrobracon* and bring it under the purview of genic balance. They proposed that sex determination in primitive Hymenoptera depended upon additive female-determining genes and nonadditive male-determining genes so that 2FD > MD > FD. Many Hymenoptera may possess this system. In *Apis* and *Habrobracon,* they suggest that the female-determining genes (*X* alleles) have lost their additivity unless heterozygous. Consequently: 2(homozygous)FD < MD > FD and 2(heterozygous)FD > MD > FD.

Kerr & Nielsen (55) presented evidence supporting the hypothesis. They found that *A. mellifera* diploid male larvae showed some female-like characters. A few other bee species (*Bombus, Trigona*) when inbred, show only females and very little inviability. In *Melipona* species which were considerably inbred, no diploid males were found. It is likely that the caste-determining alleles of *Melipona* are comparable to the sex-determining alleles of *Apis;* homozygosity for caste-determining alleles results in a sterile female rather than a diploid male.

Some of the most unexpected developments have come recently from Woyke's laboratory, and these concern the fate of the homozygous diploids. Those working with bee genetics had generally assumed that homozygous diploids simply die as do many *Habrobracon* diploid males. And indeed they do, but not in the way visualized. Diploid males hatch (148) and the larvae are eaten by worker bees (150). Woyke in a series of studies has confirmed that the "inviable" diploids are male (149) and can be incubator reared to maturity in isolation from worker bees (151, 154, 156). Homozygous mutant queens mated to one wild-type drone in each case, and producing 50 per cent "inviable" progeny, produced drones under incubator rearing reflecting the genotype of the father (153) and thus presumably diploid. Cytological investigation of eggs from queens producing 50 per cent "inviable" brood by Woyke, Knytel & Bergandy (159), showed sperms in almost all eggs from worker cells, indicating that they had been fertilized, and in slightly older eggs, the diploid chromosome number was counted by Woyke & Knytel (158). Bees do not eat diploid males because they are in worker cells (155), but at the XXI International Congress of Apiculture, Woyke (157) presented evidence of a diploid drone substance which apparently acts as a pheromone releasing eating behavior in adult workers. Thus, coincident with a sex-determining mechanism which produces the anomalous diploid drones is a behavioral mechanism which insures their elimination from the breeding population.

Eye Colors.—Probably the first genetic analysis ever done on bees with

the aid of artificial insemination was done by A. S. Michailoff, Tula, U.S.S.R., who had learned the insemination technique from Lloyd R. Watson, its developer. Michailoff (81) studied a mutation which he referred to as *white eyes* although he named it *ivory*. No genetic symbol was used. The eyes, white at emergence, turned slightly yellow later. This does not fit the description of any current white-eye phenotype but tends to resemble the expression of *tan* (s^t), an allele of *snow*. Both white-eyed drones and workers were obtained in ratios expected from a single Mendelian recessive allele.

According to Green (31), "On the basis of solubility and spectral analysis, the bee eye pigment is insectorubin". A number of mutants interfere with its synthesis. Counting the modifier (*m*) of ch^1 and omitting Michailoff's *white eyes,* there are 16 alleles at nine loci known to affect eye color. Three loci (*ch, i,* and *s*) show multiple alleles. Each of four alleles (*i, cr, pe,* and *s*) results in white-eye color. *Ivory* and *snow* in mosaic combination with wild-type eye tissue are nonautonomous whereas *cream* and *chartreuse* with wild-type are autonomous (110a). By chemical analyses and bioassays, Green (31) has concluded that *s* mutant tissue accumulates nonprotein tryptophan and that this mutant is homologous with *v* in *Drosophila melanogaster* and *a* in *Ephestia kuhniella*. Similarly *i* in bees accumulates kynurenin and is homologous with *cn* in *D. melanogaster, o* in *Habrobracon juglandis* and *w-1* in *Bombyx mori*. Mutations, in other insects, affecting ommochrome synthesis are listed in Wagner & Mitchell (141).

Expression of *bk, ch,* and *bk-ch* was studied in immature stages of drones and workers, and compared with wild type. Effects of the mutants can be detected very early in development (152).

Body color.—Natural pigmentation in honey bees ranges from black to various degrees of yellow. We recognize both color intensity and color pattern (30). In genetic analysis, the color pattern is used more than the color intensity as a phenotypic characteristic. Intensity is very often altered and is difficult to measure, although Goetze (30) gave a kind of color grading scale. It is known (126) that higher than normal brood rearing temperatures influence the color intensity but not the color pattern.

The coloration of the cuticula in natural honey-bee populations is correlated with ecological locality, latitude, and altitude. Smith (122) found that bees in African forest jungles are dark and in desert regions are yellow. Bees become blacker as one moves northward. Yellow bees are found in lowland plains, and dark bees in higher altitudes in the case of both *A. mellifera* (30) and *A. cerana indica* (85).

For a long time, attempted genetic analyses of body color inheritance have seemed contradictory and confusing (6, 27, 29, 86, 161). Multiple mating of queens, and possibly mating after initiation of oviposition (57a), contributed to the confusion. Use of artificial insemination to insure knowledge of matings is resulting in more dependable results and better understanding.

Roberts (97, see also 99) studied inheritance of black and yellow using

inbred lines of bees. Inbred yellow queens mated singly to black drones produced worker progeny that were intermediate between the parental lines; drones were yellow like the mother. Drones produced from F_1 queens showed gametic segregation and ranged from yellow to completely black, sugesting multiple-factor inheritance. Backcrossing F_1 queens to black drones resulted in worker progeny ranging from intermediate to black, whereas backcrossing F_1 queens to yellow drones resulted in worker bees ranging from intermediate to parental yellow. Roberts concluded that at least seven different loci were segregating for abdominal color in his experimental material.

Kulinčević (56) extended the analysis of body color in bees by making a detailed study of drone tergites of nine different races. The patterns of black pigment deposition could be organized into a graded series of ten classes for eight of the races. Considerable variation existed in each race, since most races were spread over most of the ten classes in a distribution that in some cases approached a normal curve, but in other cases deviated widely. The pattern of pigment deposition in one race, *A. m. ligustica* was different from that in the other eight, and so, for *ligustica,* a different series of classes was constructed. In the case of each race, intermediate forms fell between classes indicating that body color varies continuously.

For genetic analysis, two generations of brother-sister (single drone) inbreeding was done which reduced variation so much that all drones fell into one class in *ligustica,* into two in *carnica,* and into three classes in *sahariensis.* Then all possible crosses of the three lines were made reciprocally for study of drones (gametes) produced by F_1 queens. Drones from hybrid queens were classified according to a third system of ten classes because neither of the first two systems was adequate. Distribution of drones was bimodal with peaks near the parental types. Distribution of reciprocal crosses differed slightly, but not significantly, it was thought. The bimodality was not explained, but it was concluded that inheritance of body color differences in these three races is definitely polygenic.

The inheritance of the color pattern was studied also in F_1 worker bees from reciprocal crosses of *carnica* and *ligustica* (57). By the Goetze system of classification for worker bees, *carnica* workers fell into class 1, *ligustica* workers into class 9, and the F_1's into class 8. The yellow of *ligustica* was nearly but not completely dominant. The patterns were very uniform and there were no reciprocal differences.

A few major mutants are known to affect body color. The recessive mutant *cordovan* (*c*) changes black body pigment to brown. The sex-limited mutant *brown* (*ac*), found in *A. m. adansonii,* changes yellow pigment to brown in males but does not act in females even when homozygous. The *black* mutant (*bl*) results in no yellow body color regardless of the yellow polygenes that may be present (59). Jet-black drones (black thoracic hair; no bronze abdominal bands) are interpreted by Tucker & Laidlaw (139) as having most of the polygenes for black along with the *black* major gene.

Wing mutations.—Five mutants are known to affect wings. *Droopy* wings, lethal in drones, is a dominant in females and renders them flightless. It was discarded after initial studies (76, photograph in 109a). *Rudimental wing,* another dominant, appears from photographs to have removed the wings almost completely from queens, workers, and drones (34). The other three mutants, all recessives, affect wing morphology, and their effects are indicated by their names: *wrinkled, short,* and *truncate* (62). *Wrinkled* shows incomplete penetrance and variable expressivity. Both penetrance and expressivity of *wrinkled* were increased by combination with the gene for *brick* eye color. *Short* and *truncate* are each semilethal in their effects.

Eye shape.—Three structural anomalies of the compound eyes have been reported (65, 70). *Cyclops* is a condition in which the compound eyes are not separated normally, but form one crescent-shaped eye on top of the head. The highest frequency reported for any colony is about 4 per cent, and the inheritance is obscure and its study refractory. Almost the same may be said of *reduced facet number,* in which facets are usually missing from the mid-lateral region of the eye. Its frequency is increased in the presence of *brick* and *garnet,* two eye-color mutants. Genetic analysis has not been successful, and both *cyclops* and *reduced facet number* have been omitted from the list of major genes. *Eyeless* (*e*), a single Mendelian recessive, results in a lack of compound-eye facets. Several hundred eyeless drones were sterile. Dissected drones were found to have no testes. Viability is somewhat reduced.

Disease and Pest Resistance.—There is evidence of variable quality, that variation in resistance to several pests and pathogens exists in honey bees. North European black bees which were brought to the United States by the early settlers, are much more susceptible to the wax moth *Galleria mellonella* L. than is the Italian race (*ligustica*). Bees developed at Buckfast Abbey are more resistant to *Acarapis woodi* (Rennie), the mite causing acarine disease, than local bees (104). The general impression exists that some bees are much more susceptible to European foulbrood than others. Variation in resistance to American foulbrood (*Bacillus larvae* White) has been demonstrated many times (26, 46, 93, 104).

Resistance to American foulbrood has been intensively investigated. Increase in resistance under both natural and artificial selection was reviewed previously (104). After highly resistant and highly susceptible lines were established, the effort shifted to analysis of the resistance in terms of the mechanism involved, and the genetic basis of each mechanism.

At present, four mechanisms of resistance are known. (*a*) Hygienic Behavior (93, 106, 147): In hygienic behavior, dead, diseased larvae are ingested (119, 133). Wilson (146) has shown that ingested spores of the pathogen pass through the alimentary canal and are egested with the feces outside the hive. Exposure to the sun eventually kills spores. (*b*) Larval Resistance (9, 69, 111): Larvae of similar age from resistant and suscepti-

ble lines reared in the same colony differ in susceptibility. The difference is unquestionably genetic (42a, 69), but the biological mechanism and the exact genetic basis are still under investigation. Evidence suggests that germination of *Bacillus larvae* spores and growth of the vegetative stage is inhibited in the midgut of resistant larvae (8). Growth of the pathogen in the midgut and penetration of the gut wall is apparently necessary before death of the larva occurs. (*c*) Spore Straining: Apparently all bees have the capacity to remove particulate matter, like pollen grains, from nectar in the honey sac, the organ in which nectar is carried from flower to hive. Spores of *B. larvae* are much smaller than pollen grains, but Sturtevant & Revel (128) found that they are differentially removed by resistant and susceptible bees, from liquid food in the honey sac. That such differential removal is an effective mechanism of resistance is indicated strongly by Thompson's results (135). (*d*) Antibiotic Brood Food: Since 10-hydroxy-Δ^2-decenoic acid is abundantly present in the glandularly produced royal jelly (queen brood food) and since it is antibacterial (10), it is no stretch of the imagination to suppose that it might be present in different concentrations in the glandularly produced worker jellies (worker brood foods) of resistant and susceptible lines. Varying concentrations of worker jelly, from each line, in bacteriological culture medium, did indeed inhibit germination of *Bacillus larvae* spores and survival of vegetative cells, and indications are that worker jelly from the resistant line was more active than that from the susceptible line (100).

Only the genetic analysis of hygienic behavior is well advanced, and this is discussed in the next section.

Behavior genetics.—The various races of bees differ greatly in behavior, and this has been known for a long time, even though quantitative, objective studies are scarce. Ruttner & Mackensen (116), and later Rothenbuhler (108), called attention to behavior differences in reviews. Observations of Brother Adam indicate the extent of the variability (1, 2, 3); papers of Hassanein and el-Banby present types of quantitative studies that can be made (39, 40, 41, 42). Particularly interesting in the latter case is the introduction of marked bees of various races into one colony, followed by measurements of their individual activities (41). Bee behavior genetics has been initiated largely by work on brood-nest-cleaning behavior and collection of alfalfa pollen in preference to pollens of other plants.

Genetic analysis of behavior of whole colonies requires genetic homogeneity of the worker bees composing the colony. Such homogeneity can be found in inbreds, in an F_1 cross of inbreds, and, in the special case of bees, in the colony resulting from backcrossing one drone from an F_1 queen to a queen of the parental inbred line (105). Studies of such colonies are sufficient for a considerable amount of genetic analysis.

This single-drone inbred-queen technique was used to analyze the genetic basis of differences in hygienic behavior of honey bees. It has been known

TABLE II

PERCENTAGE OF SAMPLED POLLEN COLLECTORS CARRYING ALFALFA POLLEN

Generation	Average High line	Average Low line
1	Base stock	
2	40	26
3	50	15
4	66	8
5	85	18

for many years that strains of bees resistant to American foulbrood remove dead brood from cells of the nest promptly, whereas susceptible bees allow dead brood to remain in the nest indefinitely (106, 147). Evidence was obtained that the large difference between two lines was mediated by two loci. The hygienic line was homozygous for a recessive uncapping allele, which led to removal of the caps from cells containing dead individuals, and a recessive removing allele which led to removal of the dead individuals from the cells (107). This two-locus hypothesis is subject to test. If it is correct, further generations developed from backcross matings producing the four types of colonies found in a 1:1:1:1 frequency (*u//u; r//r*), (*u//u; r//+*), (*u//+; r//r*), (*u//+; r//+*) should breed true for uncapping and removing at homozygous loci.

A considerable amount of effort has been devoted to investigating various environmental factors that might affect hygienic behavior. Numbers of dead larvae (between 100 and 2000) have no effect upon rate of removal by a colony of normal size (45). Bees of any age will engage in hygienic behavior, but older bees (one to two months) are more actively hygienic during a nectar flow than during a dearth of nectar (92, 134). Dearth conditions seem to result in poor hygienic behavior (11, 84). Dead brood is removed from the brood nest much faster than from the honey-store regions of the hive (11).

A very significant development in bee behavior genetics is concerned with pollen collecting behavior, and involves selection for differences in collection of alfalfa pollen, *Medicago sativa* (77, 87, 88). Initially, Nye & Mackensen found high variation in alfalfa pollen collection by counting the number of bees loaded with alfalfa pollen in samples of foragers from 356 colonies. Three high colonies and three low colonies selected from the 356, were used to start a breeding program. The generation developed from the selected colonies is generation 2 of Table II. The table shows the progress made by selection.

By generation 4, separation of the two lines was such that no colony mean of the low line overlapped any one of the high line. An F_1 generation was tested along with the high and low lines in Generation 4, and it was found to be intermediate in alfalfa pollen collection. Backcrosses to the pa-

rental lines were tested along with the parental lines in Generation 5. The investigators found no definite segregation ratios and concluded that the differences in alfalfa pollen collection are due to several genes with mainly additive effects. A number of behavioral and physiological as well as genetic questions are under study.

Preliminary evidence suggests that there may be abundant genetic variation for cranberry pollen collection (121). There is no reason to think that variation in pollen collection by honey bees exists only for these two plants. Probably bees can be developed with high preferences for the pollen of almost any plant. Such bees would probably be of great economic value for pollination of specific crops.

Stinging behavior of bees has been considered genetically in a preliminary way. Two inbred lines differed widely in temperament (107, 108). The Brown line stung at the rate of about 1.5 stings per brief colony visit and manipulation, whereas the Van Scoy line stung at the rate of about 0.01 times per identical visit. No data were taken on F_1's, but 29 colonies from backcrosses of the F_1's to the high-stinging line by the single-drone technique described earlier, resulted in colonies ranging from the most gentle of the Van Scoy line (0 stings in 14 visits) to one colony that stung almost as intensively as the highest stinging Brown colony. Eight backcrosses to the Van Scoy line performed like the Van Scoy line itself (108). It was concluded that more than two loci underlie the differences in stinging behavior.

The experience with African bees (*Apis mellifera adansonii*) in Brazil makes the Brown line seem gentle (53). This bee was introduced into Brazil in 1956 with the intention of using it in a careful breeding program. By a beekeeper's mistake, queen excluders were removed allowing 26 of 30 colonies to swarm, and the bee is now spreading in South America.

It has a number of apiculturally desirable features, but unfortunately is very cross. The differences in temperament between Italian and African bees is under study by a method developed by Stort. Strong three-frame nuclei with a laying queen are used for tests. A small black leather ball is put before each nucleus, and the following data are recorded: (*a*) time taken to first sting; (*b*) time taken to become very fierce; (*c*) number of stings in the gloves of the observer; (*d*) number of stings in the leather ball; (*e*) time to quiet down; (*f*) distance bees follow the observer after they become fierce. Results are in Table III (53).

The hybrids performed in about the same way as Italians except in time to first sting which was intermediate, and time to become fierce which was much longer. The investigators concluded that gentleness is due mainly to dominant genes, but they had no suggestions as to the number of genes controlling this difference in behavior.

Population Genetics

Maintenance of genetic variability.—Inasmuch as most mutant genes are detrimental when expressed in the phenotype, the haploid males of Hymen-

TABLE III

COMPARATIVE FIERCENESS OF ITALIANS, AFRICANS AND THEIR HYBRIDS

	Race		
	Italian bees	African bees	Hybrids
1. Time to first sting	19.3 sec.	2.9 sec.	9.8 sec.
2. Time to become fierce	22.9 sec.	7.3 sec.	45.0 sec.
3. Number of stings in leather ball	26.1 stings	63.7 stings	31.2 stings
4. Number of stings in gloves	0 stings	39.3 stings	1.4 stings
5. Time to quiet down	149 sec.	1801 sec.	243 sec.
6. Distance bees followed observer	22.8 m.	170.3 m.	31.4 m.

optera would seem to constitute an additional screen for removal of either dominant or recessive mutants from the population. That bee genotypes contain mutant genes, and consequently variability, comparable to that of other species, can be demonstrated by determining their genetic load. This genetic load, in any species, is made up of the genes responsible for mortality, sterility, and abnormality resulting from the interactions of these genes with the environment and among themselves.

The genetic load may be composed of complete lethals or detrimentals (sublethals, subvitals, abnormals). Morton, Crow & Muller (83) developed the concept of *lethal equivalent* from an initial idea conceived by Muller. A lethal equivalent may represent a full lethal, or two genes each with a one-half probability of causing death of the zygote, or three genes with a one-third probability of causing death, etc. Since methods like *CLB* cannot be applied to bees, the genetic load of a bee population must be studied by Morton, Crow, & Muller's method which detects lethal equivalents through a regression of natural logarithms of survival values on different levels of inbreeding. Kerr (48), using this method and working with 86 queens, found a genetic load of 1.347 lethal equivalents per gamete. Because the sex alleles act as true lethals in inbred populations, the load due to them can be estimated $(1 - 1/n$, where n = number of sex alleles) and substracted from the total genetic load. In one population (75) the number of sex alleles was found to be 11 so that $1.347 - (1 - 1/11) = 0.438$.

Data obtained by Kerr (49) from three different populations of *A. mellifera* suggest that a substantial part of the genetic load involves female sex-limited genes. The possibility exists for over-dominance with these genes, but there is little evidence for it except with the *X* alleles which are obviously segregational. Most of the remaining genetic load in bees is mutational (48).

Variability is also influenced by effective population size, and consequently it is important to have an estimate of this parameter in bees. Social bees have a very stable effective population size, and the total number of

queens in an area is therefore very close to the number of colonies. Each queen represents, in her spermatheca, the males which inseminated her. Since queens are likely to be inseminated by an average of ten males (131), it suffices to know the number of laying queens. The effective population size (N) is defined as $p'/(2 \triangle p)$, by Wright (160) who deduced the following expression for sex-linked genes (N_M being the number of males and N_F the number of females):

$$\triangle p/p' = (2N_M + N_F) / 9N_F N_M$$

The following formula was thus used to estimate N in bees by Kerr (50):

$$N = 9N_F N_M / 2(2N_M + N_F)$$

If each colony is headed by a queen inseminated by ten males ($N_M = 10N_F$), then: $N = 15N_F/7$. Since each colony usually has only one queen, N_F is also the number of colonies.

Evolution of social life.—Cooperation of offspring with parents in rearing siblings, which is true sociality (145), has originated at least ten times in the Hymenoptera and only once (termites) in all other Arthropoda. A problem is posed, consequently, as to how the Hymenoptera have provided a more fertile field for the evolution of sociality than other insect orders.

An important advance in understanding the evolution of social life seems to have been made by Hamilton (35, 36, 37) who considers first the evolution of altruistic behavior in general, and then moves to the evolution of sociality and reproductive altruism found in the worker castes of Hymenoptera. Hamilton (35 p. 354) means by altruism "... an animal behaves in such a way as to promote the advantages of other members of the species not its direct descendants at the expense of its own". To select for such individually disadvantageous behavior, group selection has often been invoked. But both R. A. Fisher and Sewall Wright, according to Hamilton, minimize the role of group selection in evolution. Therefore a different explanation is suggested.

Let us suppose that altruism depends upon a given gene in a population. The probability of perpetuation of this gene by its individual possessor is low because the individual behaves self-sacrifically. This is true specifically in the usual diploid animals. In the haplodiploid Hymenoptera, however, an unusual genetic relationship prevails. If a diploid female mates with only one haploid male, Wright's coefficient of relationship between mother and daughter is one-half, between sisters it is three-fourths. Other things being equal, a female offspring would be more likely to procreate the altruism gene received from her mother if she rears an extra sister than if she rears a daughter of her own. Selection for altruism consequently is much more likely in the Hymenoptera than in regular diploid animals.

Hamilton presents a complex mathematical model and extensively discusses biological facts which stand for and against his theory. He does not minimize the role of maternal care in the transition to true sociality (see 38

p. 106). He emphasizes that failure of males to develop worker instincts is to be expected on the basis of their relationship of only one-fourth with each other and with their sisters while retaining a relationship of one-half with their daughters.

Breeding for Economic Characteristics

History of methods of honey-bee improvement has been reviewed previously (104). Striking progress was made by mass selection for disease resistance and mite resistance in more or less isolated mating yards (104). Anderson's selection for honey production gave striking results (5). Successful selection for alfalfa pollen collection has been carried out with the aid of artificial insemination. These actual achievements in bees, as well as heritability estimates on certain bee characters indicate that tremendous progress can be made by selection (94, 125). The recent reevaluation of mass selection in corn, reviewed by Sprague (127) and showing its effectiveness, is highly suggestive for bee breeding.

Problems are encountered, however, when inbreeding occurs. Both inbreeding depression and brood inviability due to homozygous sex alleles occur to the point that lines cannot be maintained under natural apicultural conditions. More theoretical and experimental efforts are needed to devise practical selection and mating systems. Soller & Bar-Cohen (125) have made one suggestion which utilizes a more or less isolated mating yard. Schemes are needed which more rigidly control matings.

Hybrid breeding is considered to be the solution to the problem of inbreeding depression and homozygous sex alleles (99). Mackensen & Roberts (79) state that certain double hybrids have produced as much as 50 per cent more honey than comparable commercial lines. Roberts (98) studied several morphological characters (wings, proboscis, antennae) in inbred lines of bees. Small but highly significant differences among lines were found, along with evidence for heterosis in some line crosses. Heritability was high for some characteristics and low for others.

Cale & Gowen (14) studied oviposition and honey yield of several inbred lines, their F_1's, and certain commercial stock. The average of the F_1's exceeded the higher parent in egg production by 35.5 per cent and in honey yield by 15 per cent. F_1's exceeded a random sample of commercial stock in egg production by 7.2 per cent and in honey production by 6.2 per cent. The several F_1's were variable; some greatly exceeded the averages.

Ruttner (114, 115) considered the opportunities for bee improvement by intraracial selection and by race hybridization. He concluded, from a wide experience and review of literature (71, 95), that very specific local adaptations (ecotypes) exist within individual geographic races and that these differences are probably hereditary. Such differences are attuned to local nectar and pollen flow conditions. Even beyond these differences are others involving characteristics which are not subjected to equally intense natural selection. Opportunities for improvement by selection are great if inbreed-

ing is kept at a low level. A number of race crosses are reviewed some of which result in heterosis and some of which do not. Ruttner emphasizes two great needs: (*a*) for caution in race crosses because of the possibility of increasing undesirable characteristics like temper in the local bees by uncontrolled hybridization; and (*b*) for preservation of the geographic races of bees as a continuing source of genetic variability. In the U.S.S.R., efforts are being made to establish natural reservations for important geographical variants (6a).

Additional Important Advances in Techniques

Regulations prohibit transportation of adult bees into certain countries because of disease dangers. In the face of such regulations, availability of races, strains, or genetic stocks of bees has been facilitated by two new techniques. Taber (130) and his cooperators shipped honey-bee semen intercontinentally by mail in capillary tubes and successfully used numerous samples for artificial inseminations. Smith (123, 124) transported immature stages of honey bees from Europe to North America.

For radiation genetics of bees (67), it is sometimes desirable to irradiate exclusively the spermatheca or the oogonia in the anterior region of the ovaries. Lee (68) has described a technique involving lead shields and a holding device, for localized irradiation which at the same time protects the ventriculus, a radiation sensitive part of the queen's body.

The necessary number of matings can be greatly reduced in tests for allelism by mixing semen from several different known mutant drones for inseminating a queen of unknown mutant type (137, 140).

The insemination technique itself is made easier by use of a vital dye to stain the vaginal tissues (16). The valve fold is seen more clearly which facilitates its depression.

The origin of male tissue of gynandromorphic honey bees from accessory sperms suggests an unusual possibility. Tucker & Laidlaw (138) have pointed out that a given genotype of sperm can be propagated indefinitely by inseminating a queen of a gynandromorph producing line with semen from one drone, followed by subsequent inseminations of other queens of the gynandromorph producing line with semen from male parts of gynandromorphs produced. By this technique, a gamete can be maintained indefinitely or produced in unlimited numbers for a variety of testing and utilization procedures. Such gamete maintenance and multiplication may be unique in the animal kingdom, and seems likely to be of value for basic problems as well as practical bee breeding.

Gamete selection is particularly accessible in bees and ought to be utilized. Furthermore, one can reclaim sperms from a killed queen's spermatheca and inseminate a virgin with them. If she is of a gynandromorph producing line, a few sperms could be increased to large numbers.

Observing and measuring behavior, disease phenomena, and morphological characteristics for genetic analysis can be burdensome in bees. Much bee

behavior is influenced by environmental conditions such as intensity and duration of nectar flow. It takes weeks for a colony to develop a large population. Many activities of the colony are hidden in the depths of the nest. Robbing of one colony by another is exasperating, and wrecks experiments.

Several steps have been taken recently to alleviate some of these difficulties (112). Observation hives have been designed and used inside a temperature-controlled, observation-hive shelter attached to flight cages in which both pollen and sirup are provided in controlled quantities. With such temperature control, colonies of 200 bees and less have been used successfully for certain experiments. The great nuisances of robbing and drifting have been eliminated, and isolation has been provided for experiments with diseases. By the use of these facilities, experimentation has become both easier and more productive.

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