

Behavioral Genetics

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I. INTRODUCTION

Darwin (1859) devoted a chapter of "The Origin of Species" to the evolution of instinct. More than a quarter of that chapter discussed the nest-building behavior of honey bees and concluded with the notion that "the most wonderful of all known instincts, that of the hive bee, can be explained by natural selection." Although Darwin's chapter might have enticed scientists to study the hereditary aspects of honey-bee behavior, it was 105 years until Rothenbuhler (1964a) provided experimental evidence for the hypothesis that the tendency of honey bees to clean their nest of dead brood, called hygienic behavior, "depends upon homozygosity for two recessive genes."

Rothenbuhler's study of hygienic behavior is a widely cited classic in the field of behavioral genetics and is the founding work of honey-bee behavioral genetics. His work, indeed most behavioral genetic work, was precluded until two scientific developments occurred. After Mendel's principles were rediscovered in 1900, a very active period of scientific inquiry resulted in the rapid elucidation of the mechanisms of heredity. In this work, geneticists followed Mendel's lead and generally chose easy access to principles through study of the variation of easily measured discrete characteristics. Behavioral characteristics generally were not studied by geneticists because these were usually thought to be primarily the product of an animal's experience. This nonevolutionary view of behavior, derived from

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the mainstream Cuvier–Watsonian traditions of psychology (Lockard, 1971), held sway until the influence of Lorenz, Tinbergen, and von Frisch (Tinbergen, 1960) brought clear focus to the Darwinian notion that animal behavior was primarily a product of natural selection and thus had a genetic basis. Genetic studies with honey bees in the last 20 years have often explored the genic contribution to behavioral variation. Nearly all of this work has been done with the western honey bee (*Apis mellifera*).

II. THEORETICAL CONSIDERATIONS

A. Regulators of Honey-Bee Behavior

All phenotypic characteristics, whether physiological, morphological, or behavioral, are the products of specific inherited potential expressed in specific environmental circumstances. The current environment, both physiological (within an individual) and ecological (outside an individual), is itself dependent upon prior genetic and environmental interactions. Behavior, the functions of which are response to environmental stimuli and modification of the environment, is particularly dependent on such epigenetic considerations. Unlike many physiological and morphological traits, behavior is quite distant from the chemical nature of its underlying genes. For example, many more physiological processes are required for a honey bee to build comb than for the production of color in her eyes. In behavior where learning (previous experience) plays a part, the stimulating environment can be considered to extend through time. Moreover, honey-bee behavior is additionally complex since natural selection has shaped it in a social context. Indeed, sociality is sufficiently strong in honey bees that they cannot live long without it (Rinderer and Baxter, 1978; Rinderer and Elliot, 1977), and when they have gone astray they seek it (Free and Butler, 1955). Thus, a honey bee's behavior is the product of its genetic potentiality, its ecological and physiological environments, the social conditions of the colony, and various prior and ongoing interactions among these three (Fig. 1).

With honey-bee behavioral phenotypes so complex in origin, it may seem surprising that productive behavioral genetic work is possible with this organism. However, since behavior is a product of natural selection it is adaptive within the context of honey-bee natural history. This topic has been extensively studied by many scientists in the last century largely because the economic value of honey bees is ultimately tied to their behavior in natural settings. Thus, honey-bee behavioral geneticists have a tremendous background of information to guide the design of their experiments in order to have adequate control of environmental and social variance. Such

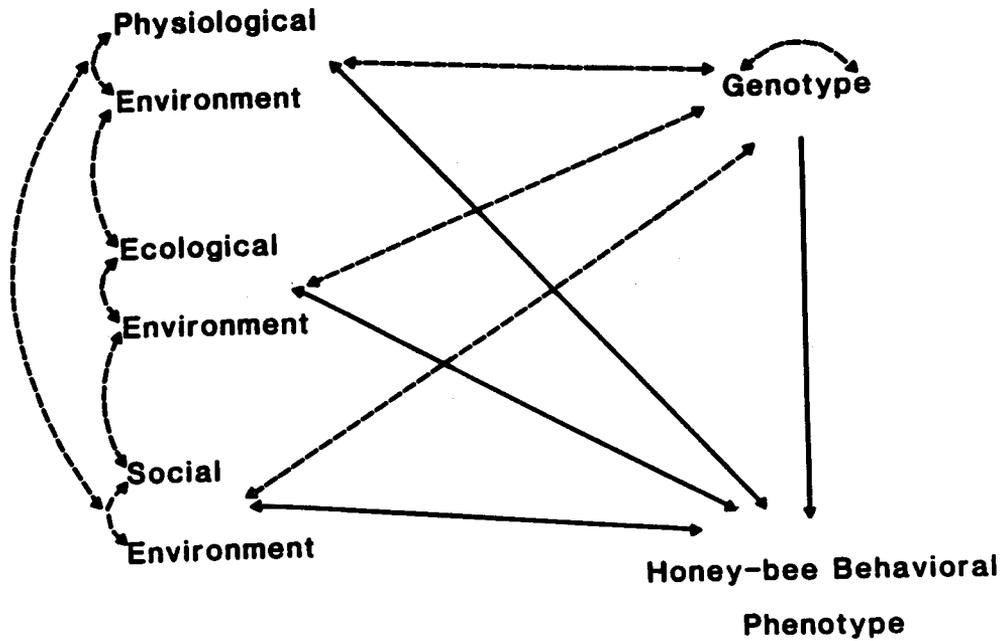


Fig. 1. The chief regulators of honey-bee behavior. Each regulator has components which interact (dashed lines) both with other components of the same regulator and with components of other regulators. This produces a complex system resulting (solid lines) in a phenotype.

designs are necessary for the productive study of behavioral genetic variance.

B. Behavioral Units of Study

Slater (1978) provides an excellent discussion of fundamental questions requiring answers before launching a research program in animal behavior. Among these questions, those involving the categorizing of behavior loom especially large for studies of the genetic aspects of honey-bee behavior. Since behavioral variation in bees has four chief interacting regulators, the kind and quality of genetic information obtained in behavioral genetic studies depends in large measure on the unit of behavior studied.

Broad units of behavior, such as nest defense or honey storage, are the products of the actions of groups of bees performed over reasonably long periods of time. Each bee performs sequences of actions which unfold according to genetic, environmental, and social regulation. The outcome of each action itself becomes a portion of the environment and greatly influences the subsequent actions of both a single bee and her hive mates. Such complexly regulated behavior will likely show a pattern of continuous variation among bee colonies. The genetic analysis of such variation is restricted to the techniques of quantitative genetics (see Chapter 11). Such analysis

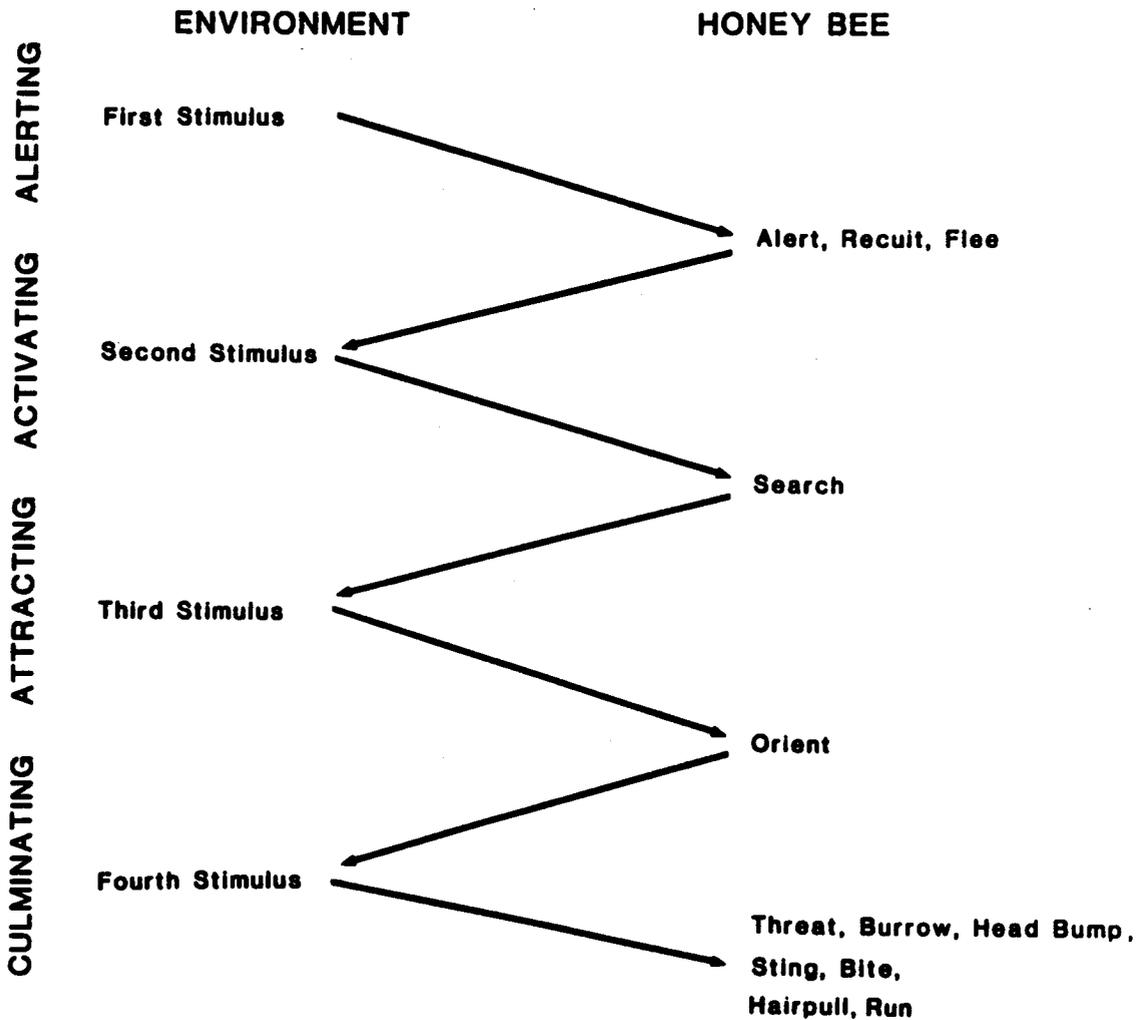


Fig. 2. Basic sequence of honey bee defensive behavior. [Redrawn from Collins *et al.* (1980). Copyright in public domain.]

will usually show that a certain portion of the variation arises from additive genetic events and that other portions of the variance are attributable to environmental and, with an appropriate experimental design, social conditions. Such information, albeit highly useful in stock improvement programs using mass selection techniques, is incomplete. Variation in early portions of the behavioral sequence arising from genetic differences may be entirely or partially masked by later events in the sequence. Also, nonadditive genetic causes of variance, as well as interactive sources of variance, are included in the variance attributed to environment (Falconer, 1960).

More information can be obtained if the behavior pattern in question is scrutinized and then described and measured in greater detail. No one method of doing so exists: both Manning (1967) and Slater (1978) suggest that units of behavior, at this stage in the development of behavioral genetics, are best resolved by an empirical approach. Nonetheless, actions

resembling the fixed action patterns studied by early ethologists, which are functionally or mechanically distinct and are repeatedly recognizable, can be used to advantage in classifying the components of complex behavior. Collins *et al.* (1980) developed a model using such units to emphasize and organize variation known to exist in honey-bee defensive behavior (Fig. 2). The attributes of the model were fitted to a measurement procedure (Collins and Kubasek, 1982) which was then used in a survey of defensive behavior variation (Collins *et al.*, 1982) and in behavioral genetic studies (Collins *et al.*, 1984).

III. CONTROL OF UNDESIRABLE GENETIC VARIATION

Honey-bee behavior geneticists have the advantage of studying a organism with wide-ranging variation in nearly all of its behavioral characteristics. Details of this variation are reviewed by Ruttner (Chapter 2) and by Rothenbuhler (1967), Gonçalves and Stort (1978), Rothenbuhler *et al.* (1968), Dietz (1982), and others. In general, variation in honey-bee behavior is noted in the differences between bees adapted to different geographical conditions. While such differences occur in good measure between subspecies, they also are known to occur across more fine-grained geographic ranges containing bees assigned to the same subspecies (ecotypes, stocks, and lines). Many of these differences remain stable when bees are moved to different environments (von Frisch, 1965; Louveaux, 1969).

As well as genetic variation between bees of different subspecies, ecotypes, stocks, and lines, substantial genetic variation exists among the members of a single colony. A normal field colony of bees is composed of a heterozygous queen which has mated to several genetically different drones, parthogenically produced haploid drones which collectively reflect the heterozygosity of the queen, and sexually produced worker bees which collectively reflect both the queen's heterozygosity and the heterogeneity of the drones with which she mated. Additionally, some of the workers may be sisters of the queen, rather than her daughters. This collection of relatives has been termed a "superfamily" composed of several "subfamilies" (Rothenbuhler, 1960).

Nearly all behavioral genetic studies with bees require the elimination of genetic variation arising from "superfamily" relationships. Most of the interesting honey-bee behavior is done by worker bees, and much of this behavior is social. Thus, techniques are required which permit the composite behavior of workers in a colony to be interpreted as arising from a common genotype. Also, behavior which appears to be individual, such as a single-bee foraging or drones and queens taking mating flights, is per-

formed in a social context that strongly influences individual behavior. For example, comb volatiles, which are products of social behavior, regulate the intensity and efficiency of an individual honey-bee's nectar foraging (Rinderer, 1982). Thus, it is desirable to assure a uniform genotype among worker bees determining the social conditions in which a behavior is measured even if that behavior is done by individual bees identified by genetic or mechanical marks.

The techniques which will control much of the "superfamily" variation are straightforward. Careful beekeeping will assure that a single queen is the mother of all the worker bees measured or contributing to the social conditions affecting measurements. Instrumental insemination, especially using only the semen from a single drone, can be used to control heterogeneity arising from the drones mated to the queen. Since drones are haploid, all the sperm from a drone are genetically identical. Thus, using the semen from a single drone assures that half the genes of worker progeny are identical. This partial identity can be enhanced when highly inbred queens are used. The technique of mating inbred queens to single drones was developed by Rothenbuhler (1960) specifically for the genetic analysis of honey-bee behavior.

By using the inbred queen-single drone technique, matings can be made which permit the behavior of entire colonies to be genetically analyzed. Worker bees from such matings will all have genotypes minimally different from the colony average. Colonies representing inbred lines differing in one or more behavioral traits can be measured as representatives of parental types. Consistent measurements suggest homozygosity for the genes regulating the traits in question. Both types of parental crosses will produce colonies of worker bees classed as F_1 progeny. An F_1 sister queen can provide genetic segregation from the F_1 generation through the drones she produces. The semen of these drones can be used to provide single drone-inbred queen backcrosses to both parental lines. The reciprocal mating of an inbred line drone to the F_1 queen is not useful since it produces a colony of heterogeneous worker bees.

One difficulty is associated with the use of the semen from a single drone for inseminations. Queens inseminated with such a small amount of semen appear to be more readily superseded and are likely to more quickly deplete their reserve of spermatozoa and cease to produce worker progeny. The selection for mating of drones that have larger amounts of sperm, careful beekeeping, and restriction of the queen to a small brood-nest area effectively reduce these difficulties. Careful insemination and beekeeping allowed Rothenbuhler *et al.* (1979) to test full-sized colonies produced by queens inseminated with the semen of single drones for an entire honey production season.

IV. APPLICATIONS OF BEHAVIORAL GENETIC TECHNIQUES

A. Behavioral Analysis of Mutants

1. Eye-Color Mutants

The detailed analysis of the effects of the mutant gene *yellow* on the mating success of *Drosophila* males (Bastock, 1956) is a major contribution to behavioral genetic literature. This study showed that the technique of mutant behavioral analysis has usefulness in revealing genetic elements in the regulation of behavior. Behavioral effects of mutants are generally maladaptive pleiotropic phenotypes attending the more apparent mutant phenotype. Detailed behavioral studies can show which precise actions in behavioral sequences are impaired and can thus also offer information on how adaptive behavior is organized and what its underlying physiological correlates are. Honey bees have several known mutants that are potentially useful in such studies.

Witherell (1972) compared the flight activity of normal drones to that of drones from 21 mutant stocks. Generally, mutant drones took fewer flights from colonies than did normal drones. This decrease was almost always associated with a shorter life span and, in some cases, with a marked reduction in the return of the mutants when they did leave colonies. One mutation, *chartreuse-red* (*ch^r*), was exceptional. Drones carrying this mutation lived as long and returned to colonies after a flight as often as normal drones. Yet they took significantly fewer flights. This result suggests that detailed observations of normal and *ch^r* drones may provide good information on the factors leading to drone flight.

Kuz'mina *et al.* (1975a,b, 1977) studied neurological and biochemical effects of *ch^r* and another eye-color mutation named *snow* (*s*). Pleiotropic effects of these two genes were found; they caused shorter neuromuscular excitability in homozygous mutant bees, an inability to orient in a flight-room, and an inhibition of dance communication following foraging. The biochemical changes characterizing the *s* mutation apparently have a neurogenic effect through the synthesis of serotonin-like compounds. Similar work by Neese (1969) with *chartreuse* (*ch*) mutants showed a reduced ability to orient to a hive or a feeding place. Also, the speed of flying and dancing was lower than in phenotypically normal bees.

2. Wing Mutants

Witherell and Laidlaw (1977) explored the effects of the recessive mutant *diminutive-wing* (*di*) on aspects of foraging by worker honey bees. Workers homozygous for *di* have wings with areas only 62.9% of normal. Generally,

these bees displayed foraging activities adjusted to the reduction in wing surface area. The wing-beat frequency of *di* workers was 22.2% more than normal. Outgoing foraging flights required 5.4% more time while returning foraging flights with nectar loads required 35.6% more time. Nectar foragers left hives carrying less food reserves and returned with smaller nectar loads. Pollen loads weighed less. Details of dance communication were the same with normal and *di* bees.

Work on a similar wing mutation, *short (sh)*, by Kuz'mina (1977) and Lopatina *et al.* (1977) revealed effects of the mutation on a dance rhythm (depressed), and neuromuscular excitement levels (also lower). By making a phenocopy (artificial shortening of the wings) they showed that the pleiotropic effects were not simply a result of the morphological defect.

B. F₁ and Backcross Experiments

1. Nest-Cleaning Behavior

Rothenbuhler's work (1964a) with nest-cleaning behavior is the most commonly referenced work in honey-bee behavior genetics and perhaps in the larger field of behavior genetics. The work is elegantly designed and provides a classic example of genes regulating behavior. The genetic work was part of a larger study of the mechanisms of honey-bee resistance to a disease of brood, American foulbrood, caused by a sporulating bacteria, *Bacillus larvae* White. Two inbred lines were developed which were resistant to American foulbrood through several mechanisms, including one which was behavioral (Rothenbuhler *et al.* 1968). Resistant bees removed dead larvae and pupae from the brood nest at a high rate and were termed hygienic. Contrasting susceptible lines were also developed which removed dead brood only slowly or not at all and were termed nonhygienic (Rothenbuhler, 1964b).

Rothenbuhler (1964a) experimentally assessed an inbred resistant line (Brown), an inbred susceptible line (Van Scoy), and appropriate crosses. Three Brown colonies were uniformly hygienic and four Van Scoy colonies were uniformly nonhygienic. This contrast permitted the hypothesis that the lines differed genetically in their regulation of nest-cleaning behavior. Furthermore, five F₁ colonies, produced by queens inseminated with the semen of single drones and reared concurrently, showed nest-cleaning behavior very close to that of the nonhygienic Van Scoy colonies. This result suggested that the difference in nest-cleaning behavior was due to recessive genes at one or more loci.

Drones were reared from two F₁ queens and 29 single-drone inseminations were made with Brown line queens. The frequency of hygienic nest

TABLE 1. Genetic Hypothesis Explaining Differences in Nest-Cleaning Behavior among Inbred Lines F₁ Hybrids, and Backcrosses*

Hygienic Brown inbred line × nonhygienic Van Scoy inbred line	<i>uu,rr</i> × <i>++; ++</i>			
Nonhygienic F ₁ F ₁ gametes as drones from F ₁ queens occur in equal frequency	<i>+u, +r</i> <i>u,r; u,+; +,r; ++</i>			
Progeny of F ₁ and recessive Brown line in equal frequency	<i>uu,rr</i> Completely hygienic, uncap and remove	<i>uu,+r</i> Uncap but do not remove	<i>+u,rr</i> Do not uncap but remove	<i>+u,+r</i> Completely nonhygienic, neither uncap nor remove

* Data from Rothenbuhler (1964a).

cleaning among these backcrosses to the line carrying the recessive genes provided information concerning both the underlying genetic mechanism and also the behavioral mechanisms of nest cleaning. Half (14 of 29) of the backcross colonies were nonhygienic and half were hygienic to some degree. Some of the hygienic colonies (six of 15) were completely hygienic while some (nine of 15) removed the caps of cells containing dead brood but did not remove the dead remains. This curious result prompted further testing of the nonhygienic colonies. Combs containing cells with foul-brood-killed brood were uncapped by the investigator and placed in the brood nests of the 14 nonhygienic colonies. Six of these colonies removed dead remains from the uncapped cells at a high rate and were classified as removers. Eight of the colonies removed dead remains at a lower rate or not at all and were classified as nonremovers. Thus, the backcross colonies fell into four classifications: $\frac{1}{4}$ uncapped cells and quickly removed the dead remains, $\frac{1}{4}$ uncapped but only slowly removed remains, $\frac{1}{4}$ did not uncap but quickly removed the remains, and $\frac{1}{4}$ did not uncap and only slowly removed remains.

These results permitted a genetical hypothesis to account for the differences in nest-cleaning behavior between the two inbred lines (Table 1). The hypothesis suggested that the difference may be due to genetic differences at two loci, with one regulating uncapping and the other regulating removal. The hygienic line was hypothesized to be homozygous for recessive alleles for uncapping (*u*) and for recessive alleles for removal (*r*). The nonhygienic line was hypothesized to be homozygous for dominant wild-type alleles (*+*) at both loci. F₁ hybrids, heterozygous for both loci, display the nonhygienic phenotype due to the effects of dominance. Backcrosses to the hygienic line

from the F_1 as it segregates through drones from F_1 queens produce four classes of colonies in equal frequency: those with worker bees homozygous recessive at both loci (uu,rr), those with bees homozygous recessive for uncapping but not removal ($uu,+r$), those with bees homozygous recessive for removal but not uncapping ($+u,rr$), and those with bees that are homozygous at neither locus ($+u,+r$).

This hypothesis explained most, but not all, of the results. Backcrosses were also made to the nonhygienic Van Scoy line. All of the resulting colonies were expected to be nonhygienic, yet one of eight contained bees that both uncapped and removed at a good rate. The measurements of dead brood removed by the non-uncapping backcrosses when given experimentally uncapped dead brood ranged from 0 to 92%. Generally, the distinction between fast removal and slow removal was clear but one or two colonies might be viewed as intermediate. Among the backcross colonies that uncapped but did not remove the contents, one colony had opened only half the cells by the end of the test. However, the distinction between uncapped and non-uncapped was more decisive than the distinction between removed and nonremoved.

Rothenbuhler (1964a) identified these anomalous data, but felt they were not significant enough to negate the hypothesis which explains the bulk of the data. Although the Van Scoy line is named a nonremover, it does, in fact, remove dead brood, but at a very slow rate. This rate is strongly influenced by environmental factors. Young bees up to 28 days of age show hygienic behavior and then become nonhygienic foragers (Thompson, 1964). However, incoming liquid food enhances hygienic behavior (Thompson, 1964) and will even cause foragers of a hygienic line to return to nest cleaning (Palmquist-Momot and Rothenbuhler, 1971). Because hygienic behavior is measured in field colonies, the natural differential of incoming nectar between colonies, possible occasional drifting of hygienic foragers to nonhygienic colonies, and colony age-structure differences may account for the anomalous data.

2. Defensive Behavior

a. European Bees. In conjunction with his analysis of hygienic behavior, Rothenbuhler (1964a) also observed differences in stinging behavior. The susceptible Van Scoy line almost never stung the experimenter during visits to the colonies, while the resistant Brown bees stung often. A common belief that disease resistance and defensive behavior were due to the same underlying character, vigor, was disproved by observations on the 29 backcrosses of F_1 to Brown line queens. Both colonies that were hygienic and those that were nonhygienic showed various levels of stinging. Analysis of the distri-

bution of stinging behavior in these colonies indicated that more than one or two loci were involved in this behavioral difference and that the tendency to sting was recessive.

Three other measures of defensive behavior were later studied using the Brown and Van Scoy lines, as was a related physiological character, production of isopentyl acetate (IPA), a honey-bee sting alarm pheromone (Boch and Rothenbuhler, 1974). Six Brown colonies, seven Van Scoy colonies and eight F_1 crosses were measured for their response to human breath at the hive entrance, opening of the hive without smoke, and IPA presented on a cork next to the entrance. Also, 25 workers from each colony were sampled to determine the amount of IPA present.

The defensive Brown line was more responsive than the Van Scoy line in all tests and produced more IPA. The F_1 hybrids resembled the Van Scoy parent in response to breath and IPA, which indicated dominance of the gene or genes for mild response. The intermediate responses of the F_1 s in the opening test indicated a lack of dominance in this character. Extreme variation in IPA production among the F_1 colonies was attributed to a complex genetic situation which probably included effects of a polygenic determination, heterozygosity in the parental lines, dominance, epistasis, and heterosis.

Two of Boch and Rothenbuhler's behavioral tests and a third, response to a moving leather target, were used by Farrell (1977) to analyze backcrosses from an inbred queen - single drone mating scheme between the Brown line and a different gentle line named YD. Backcrosses to both parental types were tested. The backcrosses to the Brown line had more bees responding to the IPA and to opening of the colony (a characteristic that Farrell calls recruitability). They were also faster to sting the leather target; yet, they delivered fewer stings than the YD backcrosses. This demonstrates the complex nature of composite colony defense behavior. Some components of this behavior are inherited differently, while others have similar modes of inheritance and probably have some common genes.

A different component of defensive behavior was studied by Collins (1979). She measured the response of caged worker honey bees to a component of their alarm pheromone (IPA) as time to react and intensity of the initial response. Initial activity level of the bees was also measured, since it affected the expression of the other characters. For all these components the expression associated with the defensive phenotype was dominant (partial, full, or over) to the less defensive phenotype. Each character difference was determined to be due to approximately two genes which were different from the genes regulating the other characteristics.

b. Africanized Bees. A series of papers by Stort (1975a,b,c, 1976, 1980) presented the results of a study of defensive behavior which measured the

progeny of queens from an Italian colony mated to drones from an Africanized colony. Backcrosses of drones from F_1 queens were made to each parental colony. This follows the Rothenbuhler (1960) scheme except that the parentals were not inbred; they were single representatives of two types of bee. Stort measured five components of the behavior: (1) time at which the first sting reached a leather ball, (2) time taken for the colony to become aggressive, (3) number of stings in the gloves of the observer, (4) number of stings in the leather ball, and (5) observer persecution behavior (or distance followed while walking away).

For two of the components, Stort proposed specific genetic systems since the distribution of behavior in the two types of backcrosses followed simple Mendelian segregation patterns. Number of stings in the gloves was inferred to be controlled by a pair of genes, F_1 and F_2 : F is the dominant gentle Italian behavior type (few stings) and f is the recessive aggressive type. Only bees with f_1f_1 , f_2f_2 genotypes were aggressive. A different two-gene system (A and B) with m alleles for gentleness and br alleles for aggressiveness was proposed to account for the differences seen in the number of stings in the leather ball. When an $A^mA^m, B^{br}B^{br}$ genotype is found, or there are more m alleles than br , the behavior expressed is gentle (few stings). Otherwise the bees are aggressive.

In the time to the first sting and the time to become aggressive the F_1 colonies were as aggressive as the Africanized type. The variation shown by the backcross colonies for these measures did not follow a simple segregation pattern, and their genetic bases were assumed to be complex and involve at least two genes for each behavior. The fifth character, persecution, was also complex. The F_1 was gentle, which indicated dominance of the gentle Italian type. However, the backcrosses to both parentals had both gentle and aggressive colonies. Stort suggested at least three genes controlling the differences here.

The disagreement between conclusions from several studies of defensive behavior serves to demonstrate the difficulty of studying a complex behavior. The different ways that the behavior was quantified caused the measurement of different components of the behavior that were determined by distinct sets of genes. Only a few of the measures were specific enough to clearly show the underlying modes of inheritance. It is possible that fine tuning of the processes of actual measurement could more clearly show the genotypes involved. However, some characteristics may be so polygenic in regulation that only quantitative approaches are possible.

3. Flight Activity

The daily flight patterns of Africanized, European, and F_1 hybrid bees were investigated by Kerr *et al.* (1970) and reviewed by Gonçalves and Stort (1978). Small but consistent differences were observed in the time of day at

which foraging flights began in the morning and ended in the afternoon. Africanized bees flew both earlier and later. Additionally, the time of peak flight by European bees occurred in the morning and by Africanized bees in the afternoon. The daily flight pattern of hybrids was similar to that of Africanized bees and suggests dominance effects in the genetic systems regulating flight activity.

4. Learning

Several simple experiments on genetic components of learning in honey bees have been done. Five colonies of different genetic origin, some of which were F_1 s of some of the others, were measured by Kerr *et al.* (1975) and found to differ in learning ability measured as discrimination and extinction during feeding at an artificial source. Ott and Brückner (1980) found that inbred lines learned color choice more slowly and had lower percentages of correct choices than did non-inbred bees. Their conclusion was that these behavioral traits are affected by inbreeding depression and are, therefore, partially genetic in origin. All of these results imply genetic influences on the behavior because the bees used differed in their genotypes. As such, these studies set the stage for more refined genetic studies of learning.

5. Hoarding Behavior

Laboratory hoarding behavior was independently reported several years ago by investigators at two laboratories (Free and Williams, 1972; Kulinčević and Rothenbuhler, 1973). Small numbers of bees in laboratory cages will take sucrose solution from a gravity feeder and place it in comb provided in the cage. This laboratory behavior, at least in certain instances, is correlated to the amount of nectar collected by bees of the same genotype in field experiments (Kulinčević and Rothenbuhler, 1973; Kulinčević *et al.*, 1974). Partially because of its economic possibilities, hoarding behavior has received considerable experimental attention.

An inbred-line F_1 experiment (Brückner, 1980) was used to determine whether or not heterotic effects were involved in the genetic regulation of hoarding. Worker bees from three unrelated inbred lines had slower hoarding rates than non-inbred workers, even though the inbred lines were not selected for reduced hoarding rate. Hybrid (F_1) workers from a mating of bees from two of the inbred lines hoarded significantly faster than bees of either parental line. These results indicate that heterozygosity at some or all of the several loci involved (Rinderer and Sylvester, 1978) enhances hoarding.

C. Selection Experiments

1. Pollen Collection

Pollen collection is a fundamentally important activity of honey bees, since pollen is their exclusive protein source. Plants producing pollen collected by bees vary in floral morphology and hence in the difficulties confronting bees during pollen collection. Alfalfa (*Medicago sativa*) flowers have a tripping mechanism which causes the sexual column to strike the underside of the head of bees collecting pollen. This mechanism, although assuring effective pollination, seems to cause bees to collect pollen from other floral sources if they are available (McGregor, 1976).

Nye and Mackensen (1965) surveyed foragers returning to colonies in an alfalfa seed-production area and found substantial variation in the percentage of pollen collecting bees that collected alfalfa pollen. From this survey they identified three colonies that had collected a high percentage of alfalfa pollen and three colonies that had collected a low percentage. These colonies were used to start a breeding program which continued through seven selected generations (Mackensen and Nye, 1966, 1969; Nye and Mackensen, 1968, 1970). Selection was effective in producing two lines differing in their pollen collection behavior within four generations (Table 2). Fifth-generation backcrosses were generally intermediate in the percentage of alfalfa pollen they collected. The variability of the response of backcrosses indicated that the character was genetically regulated by several genes with additive effects. The reduction of progress in the later generations, in combination with a substantial reduction in within-line variance, provided evidence that a selection plateau had been reached in both lines. The underlying behavioral changes that resulted in increased collection of alfalfa pollen

TABLE 2. Change in Percentage of Pollen Collectors Carrying Alfalfa Pollen Resulting from Bidirectional Selection*

Selected generation	High line (%)	Low line (%)
2	40	26
3	50	15
4	66	8
5	85	18
6	86	8
7	87	36

* Values are group averages. Base stock: 32%.

also affected the percentage of pollen collected by the two lines from several different sources (Mackensen and Tucker, 1973).

2. Hoarding Behavior

The feasibility of bidirectional artificial selection on hoarding behavior to develop stocks producing greater and lesser amounts of honey in the field was studied by Rothenbuhler *et al.* (1979). A mating design styled after the one presented in Fig. 3 was organized to reduce problems associated with inbreeding and still use an experimentally manageable number of colonies.

Results in the first selected generation show that hoarding rate increased in the fast line but did not decrease in the slow line (Fig. 4). Thereafter, good progress was made in the slow line in the second, third, and fifth generations, but little progress was made at increasing hoarding rate in the fast-hoarding line. However, the selection pressure applied to the slow-hoarding line was considerably less than the selection pressure applied to the

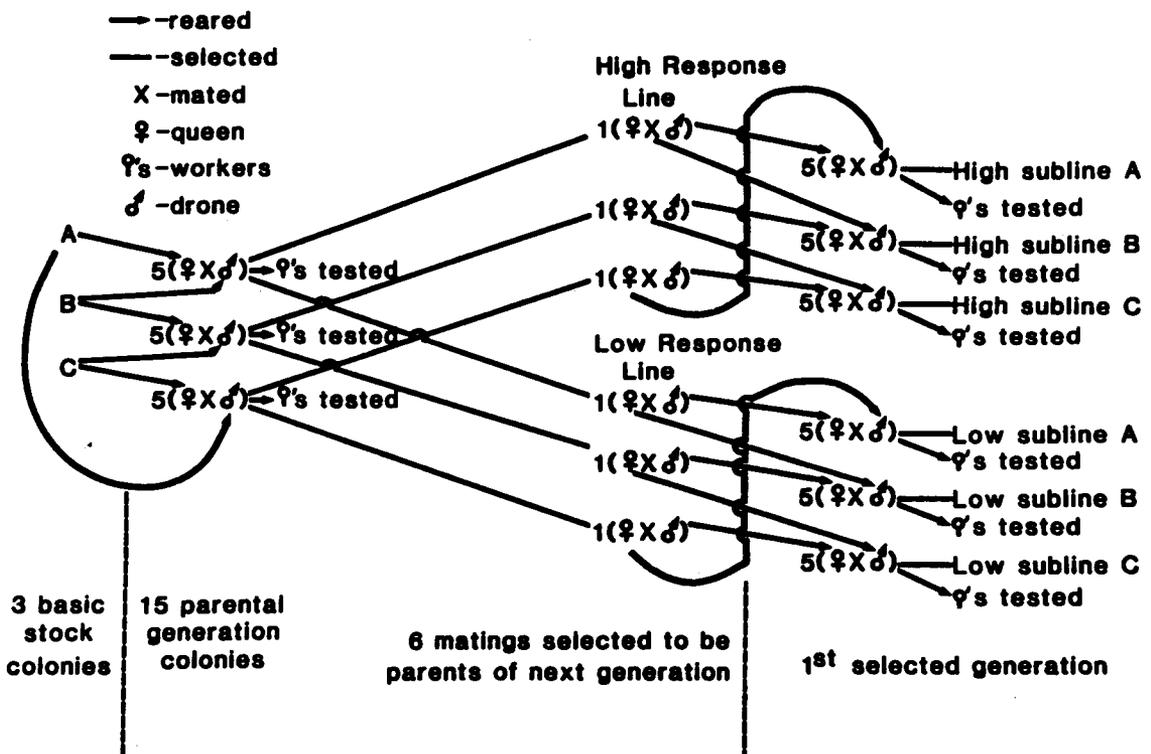


Fig. 3. A mating design reducing problems associated with inbreeding. Arrows indicate the origin of individuals; lines (without arrowheads) indicate that a selection was made among matings. Drones are haploid, and each queen was mated by artificial insemination to one drone. Progeny tests of each mating were made by testing worker bees for response. Results of these tests indicated which matings should be chosen to produce each new (selected) generation. [Redrawn after Kulinčević and Rothenbuhler (1975), with permission. Copyright 1975 by Academic Press.]

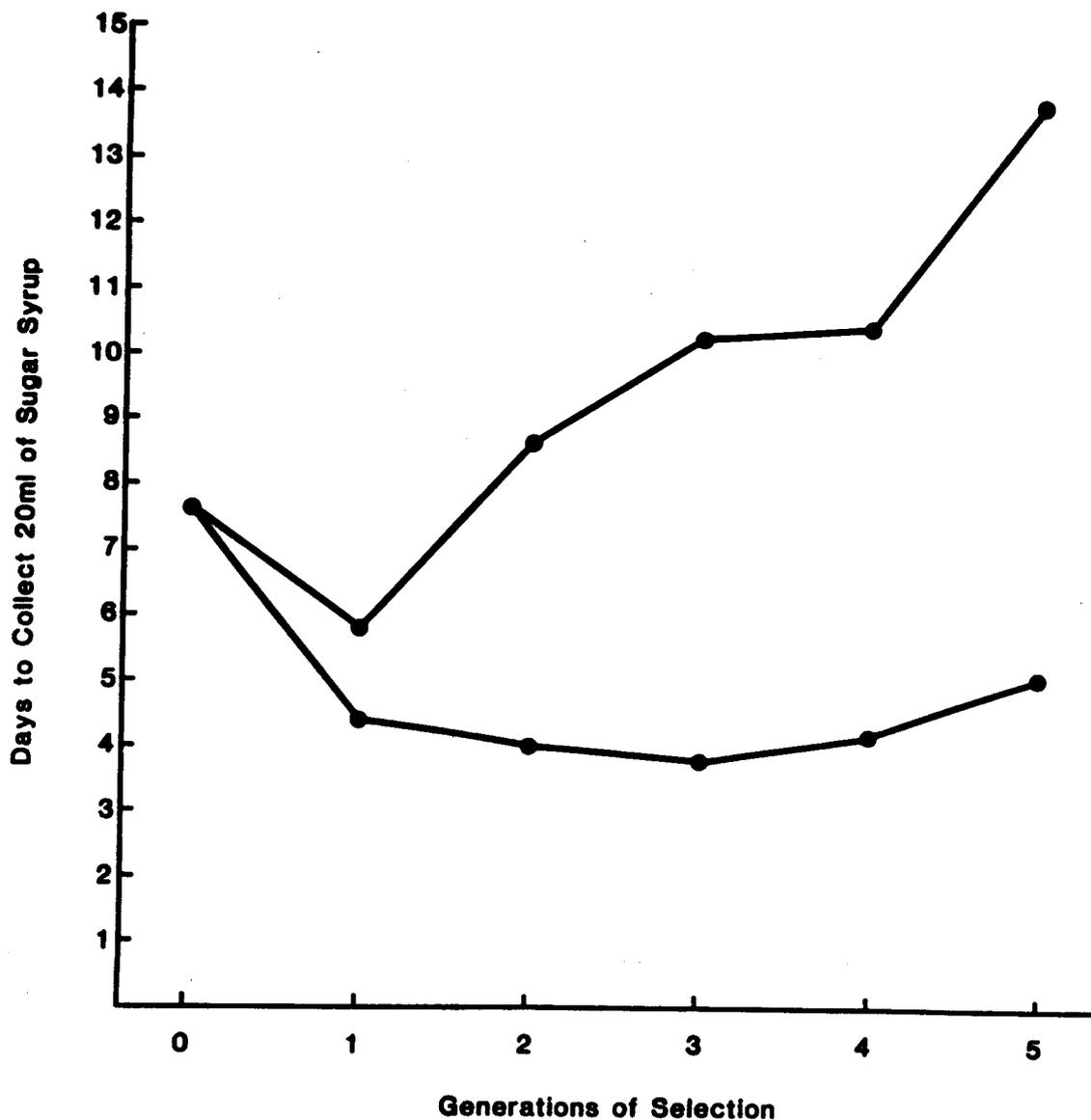


Fig. 4. Progress in selecting for fast and slow hoarding behavior in laboratory cages expressed as the time taken by caged samples of bees in successive generations to collect 20 ml of sugar syrup. Connected points are means from about 20 matings tested in each generation of each line. [From Rothenbuhler *et al.* (1979), with permission. Copyright 1979 by International Bee Research Association.]

fast-hoarding line. The hoarding rate of the fast line (4 days to hoard 20 ml of sucrose solution) is slower by at least 2 days than several other colonies tested in Rothenbuhler's laboratory. Thus, the fast selected line did not plateau in its response because a physiological limit was reached. Changes in response in the slow-hoarding selected stock and survey data from Rinderer and Sylvester (1978) suggest that additive genetic events contribute substantially to the regulation of hoarding. The plateau reached in the fast-hoarding line may indicate that genetic variation available in the base

stock was generally exhausted in the development of the first selected generation. This is especially likely since the colonies of the base population were products of another selection experiment and thus were already related.

The expression of desired correlated responses in honey production was ambiguous. Three separate field tests of the second generation showed that the fast-hoarding line stored more honey. Yet field tests of the fourth and fifth selected generations did not show such differences. Interactions of the behavioral genotypes with the various environments during the field tests of each generation probably produced these results. The effects of a piece of that environment, empty comb, which stimulates hoarding and honey production, have been shown to interact strongly with nectar flow conditions (Rinderer, 1982).

D. Correlated Behavior

1. *Correlated Flight Speed*

Drescher and Gonçalves conducted a bidirectional selection program on a morphological character, the number of winghooks or hamuli joining fore and hind wings, which resulted in a correlated behavioral difference between lines [reported in Gonçalves and Stort (1978)]. After 22 selected generations, the line selected for fewer hamuli averaged 10.6, while the line selected for more hamuli averaged 28.6. A collaborator of Gonçalves, M. C. O. Campos, measured the flight speed of both workers and drones of these lines and found that the bees with more hamuli flew faster.

2. *Defensive Behavior*

Stort (1978) calculated correlations between all his measured components of defensive behavior and correlations between each measure and abdominal color. In the Africanized backcrosses, time to first sting and time to become aggressive were positively correlated. Both were negatively correlated with the number of stings both in the ball and the gloves and with the distance bees followed the observer. The number of stings and distance were positively correlated. This is expected since shorter times, more stings, and following for longer distances are all aggressive behavior. In the Italian backcross colonies, number of stings in the gloves was correlated with distance followed; time to the first sting was not correlated to number of stings in the ball; number of stings in the ball was not correlated with the distance followed. All of these relationships reflect the differences in the way colony defense is expressed by the two genetically different groups.

There was no correlation between behavioral components and abdomen color, which was a discrete trait controlled by one gene.

The Brown and YD backcross colonies used by Farrell (1977) to test colony defense were the same colonies used by Collins (1979) to study worker response to isopentyl acetate (IPA). Rank correlations were calculated for seven characters: number of bees responding to IPA at the hive entrance, number of bees responding to opening the hive, number of stings in a leather target, time to first sting (Farrell), initial activity level, time to react, and initial intensity of the response (Collins). A fast and strong response to IPA in the cage was seen in bees that responded in large numbers to IPA at the colony entrance and to opening of the hive, but there were no significant correlations between the response by the caged bees and the stinging behavior of the colony.

A. M. Collins and H. A. Sylvester (unpublished data) found that time to respond to IPA and duration of response by caged workers were significantly correlated with hoarding of sucrose solution in those same cages. This indicates that some common basis for the two activities is likely; possibly they are related to the sensory perception of both IPA and sucrose solution.

E. Heritability Estimates and Genetic Correlations

Heritability (h^2) is a genetic parameter that represents the proportion of the variation of a phenotype that can be attributed to additive genetic variance. It is frequently used in conjunction with selection programs to predict results and to assess the success of selection. A genic correlation is a parameter estimating the covariance between two characteristics arising from common additive genes. A more detailed discussion of these parameters is presented by Collins (Chapter 11).

Pirchner *et al.* (1962), Soller and Bar-Cohen (1967), el-Banby (1969), and Bar-Cohen *et al.* (1978) reported estimates of h^2 for honey production from calculations of the regression of responses of open-mated offspring with those of the female parents or from variance components. These estimates ranged from $h^2 = 0.23$ to $h^2 = 0.75$ and collectively indicate that honey production, which is in part dependent on a variety of behavioral traits, is strongly regulated by additive genetic events. As such, selection programs designed to increase honey production have a remarkably high chance of success when compared to the prospects of genetically improving traits in other livestock.

Several of these authors (Soller and Bar-Cohen, 1967; el-Banby, 1969; Bar-Cohen *et al.*, 1978) also reported h^2 values for brood rearing. While the range of estimates was from $h^2 = 0.10$ to $h^2 = 0.90$, five of the seven estimates were above 0.30. Again, selection programs designed to improve the

trait have good chances of success. These authors also calculated genetic and phenotypic correlations between brood rearing and honey production and generally found quite high genic correlations ($r = 0.77 - 1.12$) and moderate phenotypic ones ($r = 0.34 - 0.51$). Nonetheless, since the variance of the regression was larger than the predicted improvement from a reasonable selection differential, Bar-Cohen *et al.* (1978) do not view a selection for number of brood cells as a reliable means of increasing honey production.

Collins (1979) used a different calculation system (regression of offspring on midparent from colonies produced by single-drone insemination) to calculate h^2 for the time to respond to IPA by caged worker bees. The estimate of 0.68 was high for a behavioral character. A more sophisticated sibling analysis approach was used by Rinderer *et al.* (1983) and estimated $h^2 = 0.03$ for the same character. Longevity of caged worker bees was also measured and had a $h^2 = 0.32$.

Quantitative genetic studies on the honey bee are in their infancy. The theoretical groundwork for adapting existing extensive methodology to the haplo-diploid social honey bee is limited. Investigators are also hampered by the biology of the organism that currently precludes the large sample sizes of thousands that are desirable for more accurate estimation of parameters. Nonetheless, this is an important area of honey-bee behavioral genetic investigation.

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