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GENETICS OF DEFENSIVE BEHAVIOR I

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Of all of the distinctive characteristics of the Africanized bee, the one that has received the most attention, especially in the popular press, is its defensive behavior. Reports in Africa with *A. m. adansonii* (now *scutellata*) included descriptions of the high levels of colony defense and the modifications this required in management techniques (Smith, 1958; Papadopoulo, 1971; Guy, 1972). The scientists who imported the original queens from Africa into Brazil took precautions to prevent introduction of this undesirable trait into the local population (Portugal-Araújo, 1971).

From the first, the proposed solutions to the problems of the Africanized bees have been genetic ones. Kerr and his associates intended to produce a gentle, high-honey-producing hybrid of *A.m. scutellata* with the other subspecies already in Brazil (*A. m. ligustica* and *A. m. mellifera*) (Nogueira-Neto, 1964). Even after the escape of the African swarms, Kerr suggested "the solution" was still to develop "a new race of bees" (1968). Thousands of Italian queens were produced and distributed to beekeepers in the area of Africanization to induce Italianization of managed colonies (Kerr, 1967, Michener, 1975). The report of the National Research Council Committee on the African Honey Bee recommended the development of a selected desirable stock to be used in a barrier zone to control the northward migration of the bee and perhaps elsewhere to alleviate problems already in existence (Anonymous, 1972).

There were obvious indications that defensiveness might be modified genetically. Defensiveness was often referred to in discussions of subspecies differences (Ruttner, 1975). Also, some beekeepers had exercised artificial

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selection on their managed stocks to produce more gentle bees. However, little was known about the basic genetics of this trait.

The situation in South America was favorable for genetic investigations of defensive behavior. Populations that showed clear phenotypic differences, varying from gentle to defensive, could be compared in the same location. There was also a hybridized population, the result of ongoing mating between the two general parental types, African and European, as well as the hybrids produced. The extreme variability expressed in this honey bee population, referred to as Africanized, may have been partly due to environment, for the same colonies often reacted differently at different times and places (Anonymous, 1972; Brandeburgo *et al.*, 1982; Villa, 1988). But the consistent association of African ancestry and extreme defensiveness implied that a majority of the between-colony differences were due to differences in genotype, and that the variability of the hybrid population could be tapped as a possible resource for a solution to the Africanized bee problem through genetic selection.

THE CHARACTER

Description

The term colony defense encompasses a complex sequence of possible actions performed by individual bees or, more usually, a group of bees. A theoretical model discussed in detail by Collins *et al.* (1980), presents this behavior as a response sequence by an individual worker bee (of whatever type) to stimuli in her environment. However, there are several modes of communication between workers that provide for coordination of many individuals into an effective social unit of defense. The most notable of these is alarm pheromone.

When an individual bee perceives an appropriate and sufficient stimulus, she becomes alerted. Her response may include a typical alert posture (Ghent and Gary, 1962; Maschwitz, 1964) with body raised, wings extended, mandibles open and antennae waving. The sting may also be extended. If appropriate stimulation continues, the bee begins random movement which serves to eventually bring her into proximity with the source of the disturbance. With continued stimulation from the intruder, the bee can orient to the disturbance, and finally attack. The obvious form of this attack is stinging, but threat behavior by a flying, buzzing bee is also effective. The defending worker may also bite, crawl into ears, nose or mouth, or burrow into fur or clothes.

An alerted worker may run into the colony releasing alarm pheromone from her extended sting. This recruits other bees to defend, and is the primary method of coordinating a group response. This recruiting bee may or may not be involved in later stages of defense. Perception of the original disturbing stimuli by many bees can also produce a group reaction. The searching, orienting and

attacking of defending bees may attract more workers to the location. Stings left in an intruder continue to release alarm pheromone, serving as markers for more attacks (Maschwitz, 1964).

Depending upon her physiological condition, a worker may not respond to disturbance of the colony by defensive behavior, but by withdrawal. This could occur at the initial time of alerting, with the bee reverting to a defensive sequence if provocation continues. Alternatively, a defending bee may reach a point at which she flees. In case of long-term disturbances, the ultimate defense of the colony may be to abscond from the nest site.

The bases for differences in defensiveness could lie at any stage of this complex series of activities. More defensive bees might perceive alarm pheromones or other stimuli, such as vibration, at lower levels than less defensive bees. If sensory abilities are the same, more defensive bees might have lower thresholds, that is, they show a behavioral response at lower levels of stimulation. A given stimulus could provoke faster, more vigorous responses or more bees to respond. The production and release of more, or more effective, alarm pheromone would enhance the group response. Greater ability to orient to and follow an intruder, greater propensity to respond by stinging than by threat or withdrawal, and longer maintenance of an aroused state could also be factors in increased levels of defense.

Quantification

Appropriate genetic dissection of defensive behavior should include measurement of many of these aspects of the behavioral sequence. Morphological and physiological assays would be less subject to environmental conditions than behavioral ones, but may be difficult to define, to perform, and to correlate with the end result of a defensive sequence. The more clearly discrete units within the behavioral sequence can be defined, the easier will be the genetic analysis. For a more complete discussion of these principles, see Rinderer and Collins (1986). A number of such assay systems have already been developed.

Study of the production of alarm pheromone began when Boch *et al.* (1962) identified isopentyl acetate (IPA) as an active component of the sting alarm pheromone. Another compound, 2-heptanone (2HPT), produced in the mandibular glands of the worker, was also shown to have an alarm function (Shearer and Boch, 1965). Fifteen more compounds associated with the sting apparatus were identified (Blum *et al.* 1978) and shown to stimulate an alert response from worker bees (Collins and Blum, 1982, 1983).

To measure production of alarm pheromone, workers of foraging age were collected from colony entrances and frozen. Stings, or stings and heads, from samples of ten bees from each colony were removed with forceps and collected over ice in pesticide-grade methylene chloride (with sodium sulfate as a drying

agent) in crimp-top vials. Samples of these solvent-extracts were analyzed by gas chromatography for alarm pheromone components.

Response to alarm pheromone components was measured in laboratory tests of caged young workers following the procedure of Collins and Rothenbuhler (1978). Bees emerged from brood cells in an incubator in single-colony groups over periods of 24 hours and were caged in glass-fronted wooden cages (Kulincevic *et al.*, 1973). They were given 24 hours to adjust to the cage before they were tested for the next three days. Observations were made on the initial activity level in the cage prior to testing, the speed of the response to the test chemical (time until a response is first seen), the intensity of the response based on both number of responders and the vigor of the response, and the duration of the response.

Southwick and Moritz (1985) and Moritz *et al.* (1985) measured increases in oxygen consumption following exposure to alarm pheromone. Group size and pheromone concentration up to 2.4 µg/ml influenced the level of the response. Oxygen consumption was highly correlated with colony temperament and is a good candidate for a quantitative measure of this trait.

The structures on the antennae associated with the perception of alarm pheromone are the *sensilla placodea* (Kaissling and Renner, 1968). It was hypothesized that more would be present in Africanized bees, enhancing defense by increasing the response to pheromone. The number of these present on worker bee antennae were estimated using light microscopy (Stort and Barelli, 1981) as well as scanning electron microscopy (Collins, unpubl. data).

A test of colony defense, the sequential response by a colony following disturbance, was developed by Collins and Kubasek (1982) to elicit the distinct phases proposed by the model (Collins *et al.*, 1980) and to quantify each of them. The bees were alerted by the application of synthetic alarm pheromone at the colony entrance and the time until the first defender exited the colony in the search mode was recorded. After 30s of exposure to the pheromone, a second stimulus, vibration, was applied to the colony by means of a marble propelled from a sling. This vibration enhanced the defense response. At 60s, two small (2 in x 2 in) dark suede patches (targets) were waved in front of the colony entrance by a mechanical device. The time until the first bee landed on a target and attempted to sting was measured. After 30s, the targets were removed and the test ended by blowing smoke around the colony entrance. Later, the stings embedded in the targets were counted. A series of photographs of the area around the hive entrance was taken at 30s, 60s and 90s and was used to determine the numbers of bees responding. After experience with Africanized bees, a second series of photographs was added to estimate the numbers of airborne defenders.

A number of other possible characters that could be used to quantify defensive behavior certainly exist. Electroantennagraph studies would measure response to alarm pheromone at a direct physiological level. Biochemical assays for levels of neurotransmitters could discover differences in basic excitability of

bee types. Some basic work has been done on perception (Wehner, 1971; Anderson, 1977) and orientation in honey bees (Lindauer, 1963) that could be used for genetic comparisons. There is room for expanded work on the events involved in colony defense. As the behavioral sequence becomes more finely dissected and understood, clearer analysis of the genetic control should be possible. However, considerable genetic investigation has already been accomplished.

THE GENETICS

Population

Perhaps the earliest behavioral-genetic analyses of colony defense were the comparative descriptions of the various subspecies. Ruttner (1975) describes *A. m. mellifera*, one of the European subspecies, as being frequently aggressive and *A. m. carnica* as the most gentle. Many such descriptions are to be found in honey bee literature. Numerous reports of the results of crosses between subspecies also exist (Ruttner, 1968). However, these evaluations were usually subjective observations based on beekeeper experience during normal hive manipulations and were not quantitative.

With the development of appropriate measuring systems, defensive behavior of populations (subspecies, races, stocks, lines or types) of bees can be accurately described and differences can be related to the underlying genetic structures of the population. Initial descriptions include the simple statistics of means, variances and ranges. Eventually, the frequencies of specific genes in a honey-bee population may be used in descriptions. Importantly, any such descriptions, whether simple or complex, apply only to specific populations at the time of the measurement since all biological systems are subject to constant change.

The exact genetic constitution of the spreading Africanized hybridized population is unknown because we have little information on the structure of the previously existing South American population of honey bees and extent of crossing with the African subspecies. In any case, there have been significant changes in the population resulting from its spread and requeening efforts designed to control it. For example, when the spreading population reached Venezuela and Colombia, it encountered an established feral population of European bees, probably hybrids of *A. m. mellifera* and *A. m. ligustica*. Also, the Brazilian government is reported to have distributed *A. m. ligustica* queens to beekeepers in the early 1960s (Michener, 1975). The net result is the appearance of local populations of varying genetic profiles, all of which likely include individuals which are genetically similar to each of the parental subspecies and all possible intermediate combinations (Lobo *et al.*, 1989; W. Sheppard and T.

E. Rinderer, pers. comm.). The honey bees in North America are also a mix, with *A. m. mellifera* and *A. m. ligustica* as the primary racial ancestors.

Mendelian

The classical Mendelian approach to genetic analysis is to cross two distinct phenotypes and to use the assortment of phenotypes in the F_1, F_2 and backcross generations to estimate the number of genes involved in the control of the phenotypic difference and their relationships. There are some difficulties associated with this approach in honey bees, because it is not possible to produce an F_2 generation. Because drones are haploid, they produce identical spermatozoa, so the array of segregation types in a cross of an F_1 queen and drones from an F_1 queen is limited. In addition, any one colony (the unit on which many aspects of colony defense must be measured) represents a collection of subfamilies as the queen usually mates with 7-10 drones (Taber, 1958). Each of these drones could have different genotypes ranging from more African-like to more European-like. If the queen is also of a mixed genotype and producing highly variable eggs by genetic segregation, the result is a colony of genetically diverse individuals.

This diversity complicates genetic analysis. Greater precision may be obtained by using inbred queens and inseminating with a single drone, a technique developed by Rothenbuhler (1960) for such studies. If extreme phenotypes of defensive behavior are used, useful information on the mode of inheritance of this character can be obtained. Nevertheless, different crosses could give different results if there are multiple alleles for the genes, or polygenic systems of control.

The genetic diversity within a honey bee colony complicates the genetic analysis in another way. A colony is normally composed of several "patrilines," each represented by the offspring of the queen and one of the drones with which she mated. Some recent studies have shown that at least one aspect of colony defense, guarding, is a very specialized task performed by relatively few bees (Moore *et al.*, 1987) and that colonies with guard bees that persisted in this behavior tended to be more defensive (Breed *et al.*, 1989). Robinson *et al.* (1989) have determined that guard bees are a non-random representation of the bees in a colony, probably from one or a few patrilines. These results imply that an assay of the defensive behavior of a colony may measure only a portion (one patriline) of the genotypic variability carried by the worker progeny of a queen.

Quantitative

Because defensive behavior is complex and difficult to dissect into basic units reflecting the influence of one major gene, a more viable approach to

genetic analysis of this character is quantitative genetics. This approach is directed to populations, not individuals, and to characters that are controlled by many genes that interact in both additive and nonadditive (e.g., through dominance or epistasis) ways. Descriptions of traits are expressed in statistical terms such as variance and covariance.

Heritability, h^2, is a major genetic parameter utilized in this approach. It is defined as the proportion of the variation of a character that is amenable to change by genetic selection. Estimates of h^2 are possible with appropriately designed groups of single-drone, inbred-queen matings with an array of phenotypes (Rinderer, 1977), or with measurements across a population (Oldroyd and Moran, 1983).

Heritability estimates are primarily used to predict the results of selection programs. Once selection has been practiced for several generations, it is possible to reestimate h^2 in a form called realized heritability (Falconer, 1981) to monitor the success of the program.

Frequently, a selection program will focus on several traits of importance. Thus, it is necessary to also know the correlations between the characters involved (i.e. different measurements of defense behavior). With appropriate familial relationship among the measured individuals, both phenotypic correlations and genetic correlations reflecting commonality of controlling genes can be calculated.

THE RESULTS

Population Surveys

Alarm pheromone production was measured in samples of worker honey bees from 150 colonies in Louisiana, USA, and 147 colonies in Monagas, Venezuela (Collins *et al.*, 1989). The former colonies were representative of the European less defensive type and the latter were representative of the Africanized defensive type. Twelve alarm pheromone components were measured by gas chromatography: isopentyl acetate, 2-heptanone, butyl acetate, 2-methyl butanol, hexyl acetate, 1-hexanol, 2-heptyl acetate, 2-heptanol, octyl acetate, 1-octanol, 2-nonyl acetate and 2 nonanol. A second portion of the study done with four-week-old Africanized and European workers reared in the same location measured three additional compounds, 1-acetoxy-2-octene, 1-acetoxy-2-nonene, and benzl acetate.

Isopentyl acetate, the major sting alarm pheromone component, 2-heptanone, the mandibular gland pheromone, and 2-menthyl butanol were found in greater quantities in European workers. 1-acetoxy-2-octene and 1-acetoxy-2-nonene were equivalent in the two types. All the other compounds were found in greater quantities in the Africanized bees. Greater quantities of alarm pheromone could increase defensive behavior by reaching threshold levels faster

following release, accumulating in greater quantity around the colony (thereby recruiting more bees to defend) and taking longer to dissipate after the event.

Response to alarm pheromone was also different between the two populations (Collins *et al.*, 1987a). Comparisons of workers of the two bee types using the laboratory assay of Collins and Rothenbuhler (1978) found no significant difference in the speed of the response to alarm pheromone. However, the Africanized workers responded in greater numbers and with more vigor than the European workers and continued to show a response for a longer time. This greater responsiveness may be the result of the Africanized bees normally existing at a higher level of nervous excitation as they were consistently more active prior to stimulation with pheromone.

Antennal receptors for alarm pheromone (sensilla placodea) were found to occur in similar quantities in both bee types when counted using a scanning electron microscope (Collins, unpubl. data). Stort and Barelli (1981) had indicated significantly more placodes on European antennae, but they worked only with the light microscope.

Colony defense, as measured by the procedure of Collins and Kubasek (1982), was considerably different in the two populations of European and Africanized bees tested in Louisiana and Venezuela (same colonies as for pheromone production) (Collins *et al.*, 1982). The Africanized workers responded twice as fast initially to the alarm pheromone as did the Europeans and about twenty times as fast when the visual stimulus (moving suede patches) was presented (Africanized mean = 0.3s, European mean = 9.2s). Many more bees from the Africanized colonies responded to the test stimuli. For bees photographed on the colony, the difference was about two-fold. However, there were large numbers of uncounted Africanized workers in the air harassing the experimenters, an activity that was rarely seen in bees from European colonies. Bees from Africanized colonies stung the suede targets 8.5 times more than the European colonies.

The large amount of variation found between Africanized colonies for measures of defensive behavior reflects the hybridized nature of the Africanized population. FIGURE 1 shows the range of one measure of defensive behavior, number of stings in the suede targets, for Africanized and European bees. There were some colonies in the Africanized population that were no more defensive than the Europeans. However, most of the Africanized colonies fell outside the general European range, and the most extreme Africanized colony produced four times more stings than the most defensive European colony.

Controlled Crosses

Response to alarm pheromone and other alerting stimuli has been investigated in inbred lines of European bees and their F_1 hybrids. Boch and Rothenbuhler (1974) stimulated colonies of a line with greater propensity to

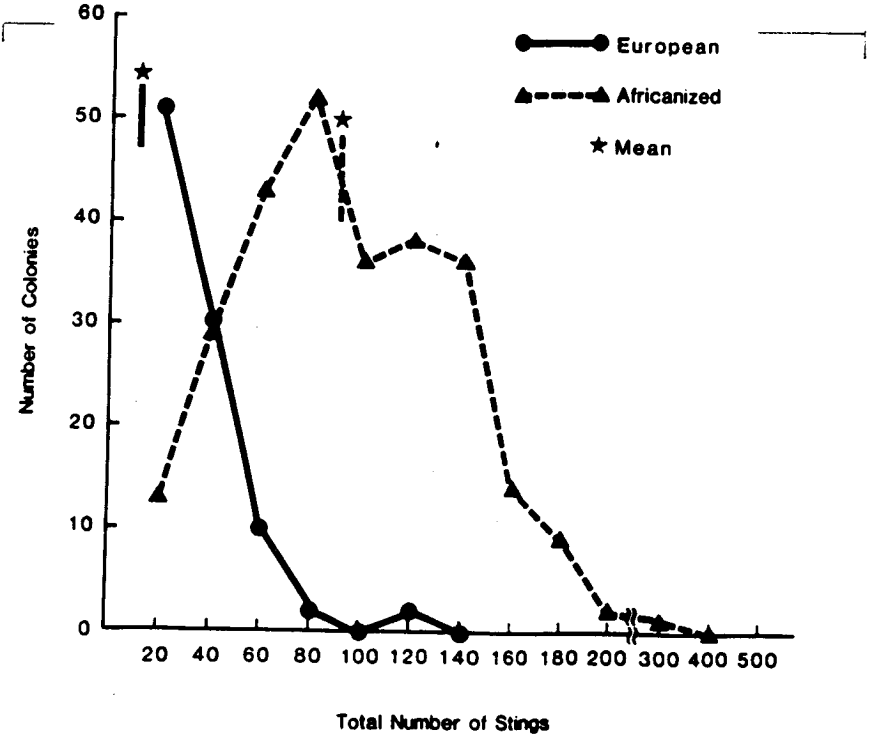

FIGURE 1. Variation in the amount of stinging by European and Africanized honey bees in a test of colony defense. Moving suede targets (2 cm X 2 cm) were presented to the colonies for 30s. (Data from Collins *et al.*, 1982. Copyright in public domain.)

sting (Brown), of one with less propensity to sting (Van Scoy), and their F_1 hybrids, in three different ways: a puff of human breath at the entrance; removal of the hive cover; and IPA at the entrance. The response of the Brown line bees was much greater than that of the Van Scoy bees. The F_1 hybrids (produced by single-drone inseminations) were similar to the Van Scoy parental type for both response to breath and to IPA, responses involving odor perception. For these two responses, the milder response was dominant. The F_1s were intermediate and different from each parental line for response to hive cover removal, a reaction more dependent on visual and tactile senses. This indicated a lack of dominance for this character.

Response to IPA by caged young workers was studied by Collins (1979) using two inbred lines, Brown-Caucasian, initially an outcross from the above Brown line and also defensive, and YD, a gentle line. Single-drone inseminations were used to produce F_1 and backcross colonies. Initial activity level, speed of response and intensity of response showed dominance of the more

responsive phenotype. For all the studies with only European bees, estimates were that two or three genetic loci controlled the line differences.

The same laboratory test of response to alarm pheromone (Collins and Rothenbuhler, 1978) was used to assay Africanized and European bees and their F_1 hybrids (single-drone inseminations) reared in Venezuela (Collins *et al.*, unpubl. data). For these crosses, the F_1s tended to be intermediate. This was different than the results just discussed for European lines. Probably different alleles or loci produced the phenotypic variation between Africanized and European lines, than produced the variation between European lines.

Production of alarm pheromone was evaluated by Boch and Rothenbuhler (1974) in the Brown and Van Scoy lines. The F_1s resembled the higher-producing Brown line, and in fact, produced greater amounts of IPA in some colonies. Because of high variability in the F_1s, the conclusions were that IPA production was controlled by many genes and perhaps the genes for increased production were different for the two lines.

The differences in stinging behavior between the Brown and Van Scoy lines were what initiated the genetic investigations into defense using these lines as subjects. The Brown colonies stung the researcher 1.5 times per visit, the Van Scoy 0.01 times per visit (Rothenbuhler, 1964). Since the F_1s were highly variable, probably two or more loci were involved.

More information is available on hybrids between Africanized bees and European subspecies for colony defense (Collins *et al.*, 1988). Colonies of Africanized bees showing extreme African-like behavior, two European subspecies (*A. m. ligustica* and *A. m. caucasica*) and two F_1 hybrids (*A. m. ligustica* x Africanized and *A. m. carnica* x Africanized) were tested with the Collins and Kubasek (1982) procedure. Speed of response to IPA clearly showed dominance of the slow-reacting parents, similar to Boch and Rothenbuhler's (1974) results. The hybrids were also more similar to the European types in number of bees around the entrance early in the test and were intermediate for total stings (FIGURE 2) and number of bees in the air following presentation of the moving targets. However, the two hybrids were different for the other characters. The *A. m. ligustica* cross produced colonies more like European parents, the *A. m. carnica* cross was more like the Africanized type for number of bees in the air early in the test (30s and 60s). The results indicate a polygenic mode of inheritance for most aspects of colony defense, and the presence of some different alleles in the two European subspecies. Kerr (1967) reported hybrids of *A. m. ligustica* and *A. m. scutellata* to be more like the Italian parent in temperament, however.

Instrumental inseminations of inbred queens by single drones from inbred lines to produce a variety of F_1 hybrids between European and Africanized bees provided further support for the hypothesis of polygenic inheritance and multiple allele differences in different lines (Collins *et al.*, unpubl. data). The phenotypes

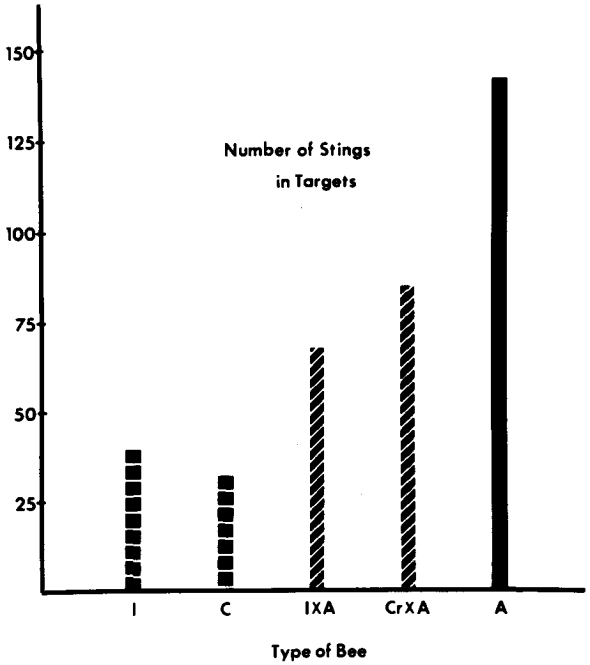

FIGURE 2. Mean number of stings in two moving targets presented to colonies of Africanized, European and hybrid honey bees. I - Italian, C - Caucasian, I x A - Italian x Africanized, Cr x A - Carniolan x Africanized, and A - Africanized. (Data from Collins *et al.*, 1988. Copyright in public domain.)

of the F_1s were similar to one or the other parent or were intermediate to the parental types depending on the specific matings.

Heritability and Correlations

Estimates of heritability have been made for components of defensive behavior (TABLE 1). Collins (1979) used regression of offspring on parent to calculate h^2 from crosses between two inbred European lines and Moritz *et al.* (1987) used the same procedure for matings with non-inbreds. Sib analyses using the sire component of the analysis of variance were used to calculate h^2 from an incomplete diallel test-cross system with European bees (Moritz *et al.*, 1987) and from an array of inbred-queen, single-drone matings with related drones (Rinderer et al., 1983; Collins *et al.*, 1984, 1987b). An alternate approach to calculation of h^2 has been proposed by Oldroyd and Moran (1983) but has never been applied to defensive behavior.

TABLE 1. Heritability (h^2) values for honey bee characters associated with colony defensive behavior.

Character	$h^2 \pm$ standard deviation	Source
Alarm pheromones		Collins *et al.*, 1986
butyl acetate	1.94 ± 0.66	"
isopentyl acetate	0.57 ± 0.55	"
hexyl acetate	1.98 ± 0.66	"
1-hexanol	0.89 ± 0.82	"
2-heptyl acetate	0.67 ± 0.57	"
2-heptanol	0.54 ± 0.54	"
octyl acetate	0.48 ± 0.52	"
2-nonyl acetate	0.96 ± 0.62	"
2-nonanol	1.07 ± 0.64	"
2-heptanone	0.59 ± 0.54	"
Response to alarm pheromones		
initial activity level	0.04 ± 0.01	Collins *et al.*, 1984
time to react	0.83 ± 0.01	"
	0.30 ± 0.01	Rinderer *et al.*, 1983
	0.68	Collins, 1979
Colony defense		
time to respond to targets	0.38 ± 0.19	Collins *et al.*, 1984
number of stings	0.57 ± 0.24	"
number of bees responding at entrance		
pre	0.26 ± 0.25	"
30s	0.12 ± 0.00	"
60s	0.14 ± 0.00	"
90s	0.55 ± 0.02	"

TABLE 2. Phenotypic correlations between selected aspects of defensive behavior, and with several other traits.

trait 1	trait 2	correlation	source	bee population
response to alarm stimuli (IPA, breath, hive opening)	IPA production	NS	Boch/Rothenbuhler 1974	European inbred lines
stinging	hygenic behavior	NS	Rothenbuhler 1964	"
initial activity level	speed of response to alarm pherome in cage	-0.49	Collins et al. 1984	European; Africanized
speed of response to target or pherome	total stings	-0.51 -0.42	" "	" "
speed of response to pheromone by colony	no. bees responding after 30s (pheromone present)	-0.86	"	"
no. bees responding at 90 s (after target presented)	total stings	0.41	"	"
no. bees responding at any time in test	no. bees responding at any other time	.60-.68	"	"

TABLE 2. Continued.

speed of response to pheromone in cage	hoarding	-.70	"	Africanized
speed of response to pheromone by colony	duration of response	-.66	Rinderer et al. 1983	European
"	response to *Nosema apis*	NS	"	"
"	longevity	NS	"	"
IPA production	no. bees responding at 90s	0.27	Collins et al. 1986	European; Africanized
2-heptyl acetate production	"	0.21	"	"
2-nonyl acetate production	"	0.21	"	"
10 sting alarm pheromones	with other alarm pheromes	0.21-.92	"	"
octyl acetate	2 heptanone	0.57	"	"

Because of haplo-diploidy and the social nature of the honey bee, traditional quantitative genetic theory based on diploid systems is not directly applicable to the honey bee. All the authors mentioned above have made attempts to apply this theory with appropriate modification in design. However, these may not be the most accurate estimates possible. Until the theory is more carefully redrafted, these stand as our best estimates. All the values in TABLE 1 except the ones for initial activity level for response to alarm pheromone and for number of bees responding at the entrance prior to the test and at 30s and 60s, indicate sufficient genetic influence on the variation of this trait to encourage attempts to select for reduced levels of defensive behavior.

Another parameter that is important in planning a successful selection program is the correlation between traits under selection. TABLE 2 presents a number of significant correlations that are known for defensive behavior. All the measures of defensive behavior are correlated in such a way that more defensive levels of one are associated with more defensive levels of another. IPA production was not correlated with response to IPA, but was correlated with another aspect of defense, number of bees responding at 90s, which involves release of alarm pheromone during stinging of targets in the last 30s.

The two traits related to disease, hygienic behavior and response to *Nosema apis*, were included in the table because it has been suggested that both defensiveness and disease resistance are aspects of basic vigor in honey bees and should therefore be correlated. These traits were not significantly correlated, however.

In the Africanized population, a fast response to alarm pheromone was correlated with a high level of hoarding, possibly because of the commonality of chemical perception in both traits. This could prove to be a problem if selection were needed for high hoarding and low alarm responses. We infer from the positive correlations of the defensive behavior components that selection for this overall phenotype should be uncomplicated by conflicting correlations. That is, selection for slower responses to moving targets also selects for less stinging, since both are less defensive phenotypes.

Selection

Based on the predictions made from the h^2 estimates for aspects of colony defense, a controlled selection program was initiated using instrumental inseminations of Africanized bees (Collins, 1986). Selection was bidirectional, for more and less defensive bees. Comparisons were made to representatives of the base population for each generation. Defensive behavior was assayed by the responses of full-sized colonies. All the measures were standardized by a Z transformation and combined into an index (I).

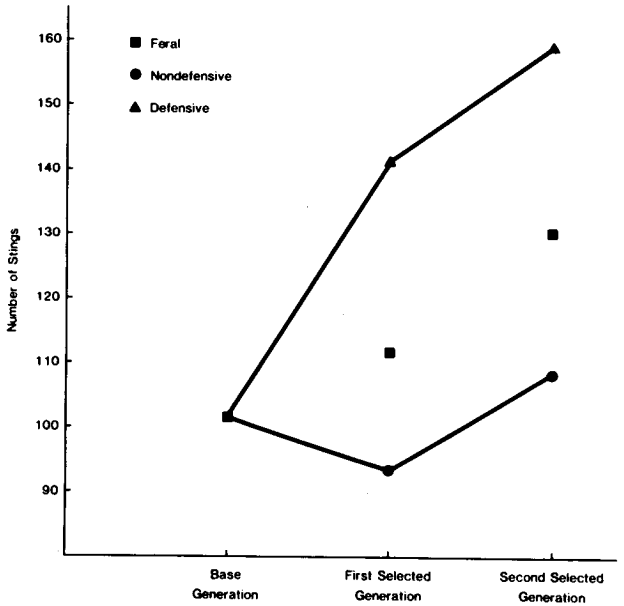

FIGURE 3. Results of a bidirectional selection program for defensiveness in Africanized honey bees. Trait presented is number of stings in two moving targets presented for 30s.

$$I = 1/2\ p + t - \frac{(30s + 60s + 90s)}{3} - s,$$

where p = speed of response to pheromone; t = speed of response to target; 30s, 60s and 90s = number of bees responding at each time; and s = total stings. The ten most defensive and ten least defensive colonies were used as parentals for each generation from a pool of approximately 80 colonies.

FIGURE 3 shows the average number of stings for the base population and the two selected generations. Most of the third generation colonies were lost to insecticide. The selection for a more defensive phenotype was progressing faster than for a more desirable gentle phenotype. The realized h^2 values 0.87 and 0.10 respectively, emphasize these results.

SUMMARY

It is clear that defensive behavior is a complex character for genetic analysis. Even when it has been divided behaviorally into some of its component parts, estimates are for several genes involved in regulation of the variation. This trait must be treated as a polygenic one and dealt with at a population level by quantitative methods (h^2 and correlations). Specific crosses of individual colonies give highly variable results, as do crosses between stocks, lines or types. However, appropriate selection programs should prove successful in reducing extreme colony defensiveness.

Discussions of Africanized bees often imply that these colonies all exhibit the extremely high levels of defense that enhance the reputation of the African, *A. m. scutellata*, subspecies. In fact, the population of bees to which the term Africanized applies is variable in genotype and phenotype. There are some Africanized colonies which are not more defensive than European types, and a greater number that have intermediate levels of defense. Kerr (1968), in an early report from Brazil, indicated that 10% of the assayed colonies were gentle, 60-70% were intermediate and 20-25% were highly defensive. It is the smaller number of colonies exhibiting the extreme of the phenotype that causes most problems for beekeepers and the public.

Beekeepers, especially queen breeders, can cope with this problem in a number of ways. In addition to attempts to maintain partial reproductive isolation for desirable, gentle stocks (Hellmich *et al.*, 1988), a continuing selection program to destroy or requeen the most defensive colonies and rear queens and drones from the least defensive colonies can be readily practiced. Use of stocks that are less defensive, and that produce less defensive hybrids when crossed to Africanized bees should be encouraged.

Further work on the genetics of defensive behavior is desirable. Other assay techniques could be found, especially some that function at basic physiological levels that are less affected by environment than gross behavioral assays. Easily performed assays that could be used by many beekeepers would be valuable. More effective, efficient production of mated queens could be achieved if there was a way to assay queens and drones directly for this trait or for a highly correlated trait. The possibility also exists that single gene mutants might occur which alter or interfere with the normal sequence of colony defense, such that massive stinging did not occur.

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