AFRICANIZED HONEY BEE

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Introduction

Background

African bees were imported into southern Brazil in 1956 and incorporated into a stock development and testing program. The aim of the program was to improve the quality of honey bees in Brazil that had originally been imported from Europe and were not productive in tropical environments. African bees were believed to be superior honey producers in tropical environments but are fiercely defensive and have numerous other objectionable qualities that make them poor commercial bees. However, it was believed that the traits of more gentle and manageable European bees could be combined with the tropical adaptedness of the African bees and the objectionable characteristics of each selected out.

African queens and drones were produced and distributed to cooperating beekeepers throughout southern Brazil as part of a stock testing program initiated in 1957. The program continued until 1962-1963 when beekeepers began complaining that their bees were getting very defensive. At that time, researchers in Brazil realized that a successful feral population of African bees had become established in an area the size of Great Britain where feral European bees had been relatively unsuccessful -- a consequence of their production and distribution program. Since that time, the feral, African population has spread throughout South, Central, and North America.

The Problem

These so-called Africanized bees (so named because of the supposed hybridization between the bees of African and European descent) created serious problems for the beekeeping industries of South and Central America and minor public health problems. (Estimates of numbers of people killed by Africanized bees since 1957 vary considerably. The **last one** I saw was 600. I believe this is minor considering people in the US die every year from stinging episodes of European bees.) The beekeeping industries of South and Central America suffered a severe decline because of the needed changes in beekeeping practices and because of reduced honey production with Africanized bees. These industries are primarily based on honey production.

The problems that Africanized bees will pose for California are different and potentially more severe because of the dependence of large sectors of California agriculture on honey bee pollination. Beekeepers transport bees in and out and up and down the state to provide pollination services to growers. Africanized bees are very sensitive to disturbance and will be difficult to maintain under these conditions. In addition, public concern over the potential public health problems associated with transporting bees will provide a political climate that will discourage the industry and lead to a severe shortage of bees that will be available for pollination.

Current Status

In recent weeks, numerous swarms of Africanized bees have been collected in southern Texas. It is clear that the population is expanding its range into the United States with the spring swarming season. Although there are indications of considerable hybridization taking place along this front of population expansion, many colonies have characteristics that appear "pure" African. The Texas expansion represents the "eastern" front. The "western" front is separated from the east by the high central plateau of Mexico and is moving along the Pacific coast. It is now expanding into the Mexican state of Sinaloa. Both fronts are about 1000 miles from California and should take 3 - 5 years to reach our border. It is conceivable that migratory beekeepers from Sinaloa could transport bees close to the border and result in "point source introductions" into California, even this year. However, it is doubtful that such introductions would have any significant effect on resident California honey bee populations. Point source introductions are probably only of political significance.

Program History

We are entering our fifth and final year of this project. There were no research activities during the first two years while a search was conducted for someone to fill a faculty position dedicated to this research. I have been in this position for two years.

Program Philosophy

There are no "solutions" to Africanized bees. Africanized bees will spread to cover the ecological range of their genotype. The process of Africanization is an interesting biological phenomenon because it is a case of one genotype of a species replacing another, even though there is no apparent reproductive isolation. They cannot be exterminated without exterminating all bees, which is probably an impossible task, certainly an undesirable one, so we need to learn to live with them and improve them for commercial practices. My research is directed toward helping to develop management programs that will replace current practices and minimize the impact of Africanized bees on California agriculture. My focus is on breeding and genetics, however, they will play a role only through the production of superior honey bee stocks, regardless of pedigree or origin, that are used by commercial beekeepers. Commercial beekeepers will only use these stocks if there are economic incentives to do so. Therefore, this is as much a problem of economics as it is genetics and breeding.

Program Objectives

I have initially established seven program objectives to guide my research:

Objective 1: Develop methods to maintain and produce commercial bee stocks that are free from the influence of Africanization. European bees are ideally suited for commercial beekeeping in California. Therefore, a primary objective of my research program is to develop methods to produce and maintain European bees in California for as long as possible. This will be particularly important for the first few years after Africanization begins while the industry and the public adapt.

Objective 2: Develop programs that will allow selective breeding and stock improvement of resident honey bee populations following Africanization. Once Africanization occurs throughout California, it will be important to have breeding programs directed toward improving them for commercial use. It will be important to know the extent and range of Africanization in the feral honey bee population of California in order to establish a viable program.

Objective 3: Determine the efficacy of and develop methods for improving the genetic composition of feral honey bee populations following Africanization. Again, we need to know the composition of the feral bee population of California before and after Africanization occurs before we will know how to proceed. It is likely that California will be a mosaic of Africanization with some areas strongly Africanized and some European.

Objective 4: Develop improved methods for analyzing DNA in order to determine the range and degree of Africanization throughout California. Initially this will be useful to reduce misidentification of European bees as Africanized. Current identification methods use morphometric tests that are size dependent. Southern California, desert bees, are small and likely to be misidentified. This objective develops needed technology to accomplish objective 3.

Objective 5: Develop better breeding techniques including instrumental insemination. Breeding is based on simple principles: 1. accurate measurements or observations of traits, 2. selection of superior performing parents, and 3. controlled mating. In order to get bee breeding, rather than just queen production, to be a component of the beekeeping industry we must develop techniques that can be readily implemented. Ultimately it will be the breeding efforts of beekeepers, not universities, that make a difference.

Objective 6: Develop new apicultural practices for commercial beekeeping. This objective includes breeding bees for specific economically important characteristics and developing new economically feasible management programs for implementing the use of selected stocks.

Objective 7: Conduct research that will contribute to a better (more informed) understanding of Africanized bee biology and behavior. Much of what we think we know about Africanized bees is based on anecdotal information. Although many of the anecdotes have been supported by scientific studies, much misinformation still persists. We need to have an informed public and industry.

Research

Selective Breeding for Increased Drone Production

This research project directly addresses program objectives 1, 2, and 3. Honey bee colonies produce reproductive queens and males (drones). Drones are produced by the thousands during the spring and summer while most feral colonies produce only 1 - 3 new queens. Queens and drones mate while in flight within a few kilometers of the nest. Queens mate with about 17 different males; males mate once and die. Feral drones are probably in competition for queen matings with drones from commercial apiaries. Therefore, any program that depends on successfully controlling the mating of desirable stocks, either for stock production or improvement of the feral population, must be able to produce large numbers of drones in order to control natural mating. One hypothesized advantage of Africanized colonies over European is their increased drone production capability. Therefore, we designed and conducted an experiment to determine if, and how, European stocks of honey bees could be selected to increase their output of drones.

Daughter virgin queens were raised from a single queen mother who had been instrumentally inseminated with the semen of a single drone. Four additional queens, designated A, B, C, and D, were selected to be drone mothers. Each daughter was instrumentally inseminated with the semen of four different drones resulting in five mating type groups: All A, B, C, or D drones and one drone from each of the A - D drone mothers. Forty-four colonies composed of workers from these crosses were then evaluated for comb construction (honey bees construct different kinds of comb for rearing drones and workers), worker population, weight of workers, and quantities of drone and worker brood.

Results from this study demonstrate that drone brood rearing is a selectable trait. It involves selecting indirectly for increased colony population and a greater proportional investment of adult workers in drone comb construction. Associated with a proportional increase in drone versus worker brood production is a decrease in the average weight of new adult workers. Therefore, drone production (as a proportion of total brood production) can be increased by increasing colony population, a selectable trait, and by selecting for an increase in the ratio of drone comb to worker comb constructed (see Fig. 1). Selection for comb construction would be very difficult for commercial bee breeding.

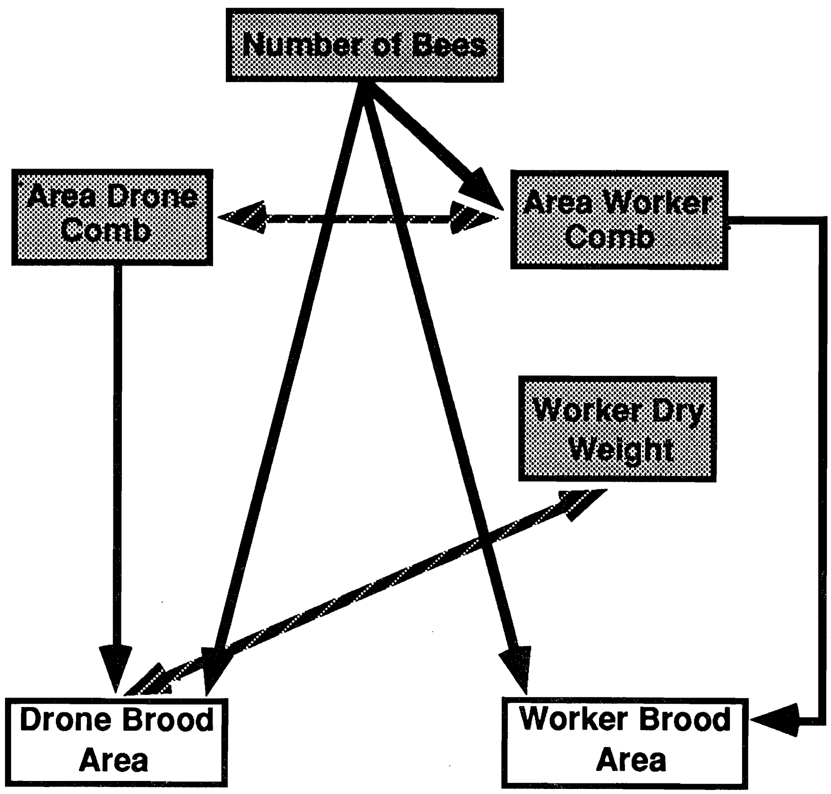

Figure 1. A developmental regulatory model of drone and worker brood production. Shaded boxes indicate model components that have demonstrated genetic variable, and are assumed to be selectable. Arrows connect components that are significantly correlated. Solid arrows are positive correlations; broken arrows are negative. The direction of single headed arrows suggests causality.

Selective Breeding for Increased Pollen Collecting

This project address objectives 1, 2, 5, and 6. We employ a closed population breeding system using instrumental insemination of queens that results in genetic isolation of the breeding population, a necessity for operating an Africanized bee free program in an Africanized area. We employ instrumental insemination and queen rearing technology that we continue to improve to facilitate the breeding program. We chose to select for pollen collecting because it is an economically important trait that will be important when California has Africanized bees.

A breeding program was initiated in the spring of 1990 that was designed to produce colonies of bees that have greater numbers of pollen foragers. Two way selection was initiated using the quantity of pollen stored in combs within the hive as the selection criterion. Four hundred commercial colonies located in almond orchards near Winters, California were examined to determine colony strength (an estimate of the numbers of workers). Of these 125 of roughly equivalent strength were measured for areas of stored pollen (see Fig. 2).

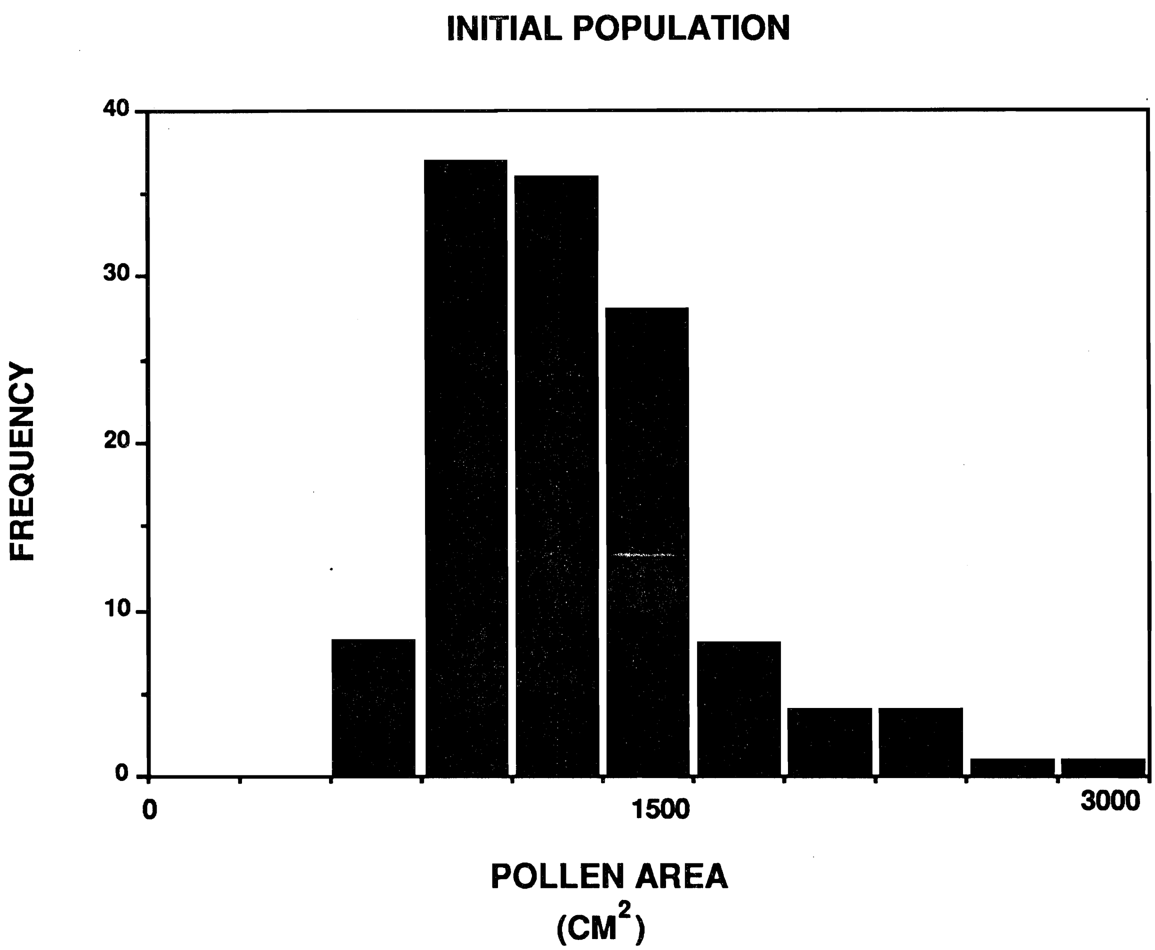

Figure 1. The frequency distribution of area of stored pollen in 125 commercial hives measured in March 1990. High and low line parents were selected from this distribution to establish the two way selection program.

Ten high-stores colonies and ten low-stores colonies were selected from them to be parents for the high and low pollen hoarding lines, respectively. Virgin queens and drones were raised from each queen of selected colonies and crosses were made by instrumental insemination to establish five sublines of high and five sublines of low pollen hoarders. These represented the first selected generation. A total of 51 colonies, approximately 5 colonies of each of the 10 sublines, were moved to the University of California Davis Arboretum and evaluated for stores of pollen (Fig. 3).

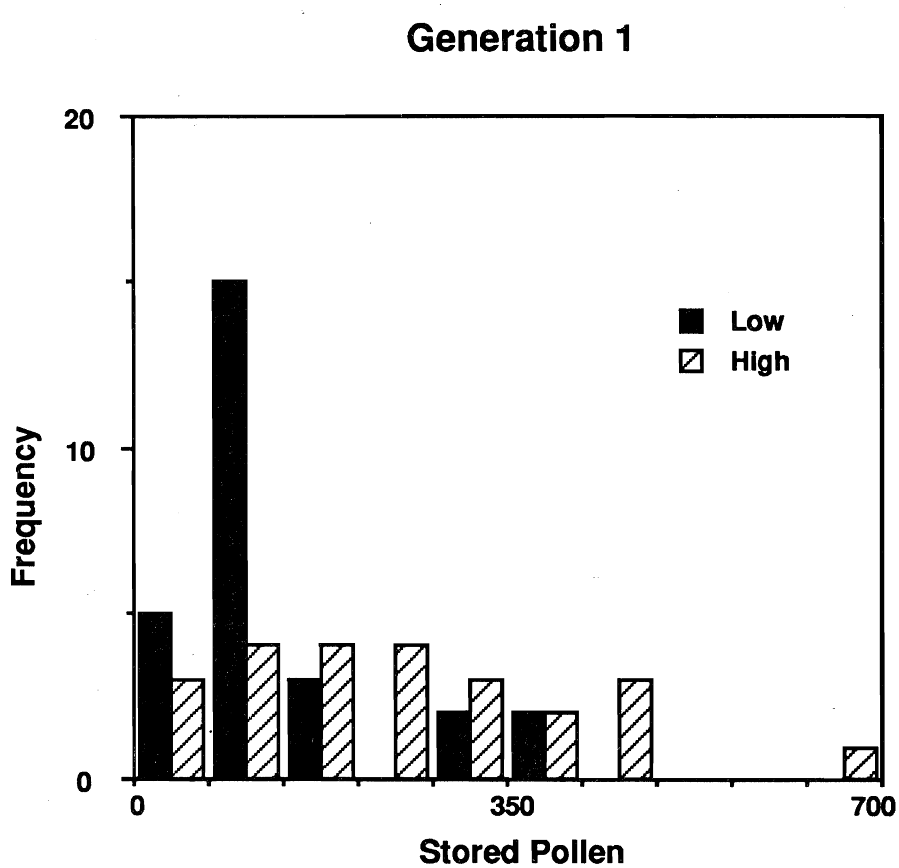

Figure 3. The number of colonies (frequency) of the high and low pollen hoarding strains that had different measured amounts of stored pollen (in cm^2) after one generation of selection.

In a single generation, high line colonies had more than two times the quantity of stored pollen when compared to the low line colonies. Colony strength and the area of brood were also estimated. There were no differences between high and low line colonies for those variables. Observations were made of foraging activity and the proportion of returning foraging bees that carried a load of pollen. The numbers of foragers did not differ between the lines but the numbers and proportions of pollen foragers did. The high line colonies had 18% more pollen foragers and a 29% higher ratio of pollen to nonpollen collectors. Nonpollen collectors were sampled and shown not to differ between high and low lines for the relative proportions that were empty, carrying nectar, or water.

The single best performing parent colony (high or low pollen stores) was selected from each subline to be parents for generation 2. Crosses were again made between sublines within high and low lines and about 8 colonies of each of the 10 maternal subline source were established to represent selected generation 2. Forty-nine of these colonies were moved into an almond orchard near Davis, California and were evaluated in March, 1991. Adult worker populations were not different between high and low line colonies and there were no detectable effects of the lines on the quantity of brood being reared. High colonies stored more than four times the quantity of pollen and had about 35% more pollen collecting bees (Fig. 4). The ratio of pollen collectors to non-pollen collectors was 30% greater in high colonies but there were no differences in the total number of foragers.

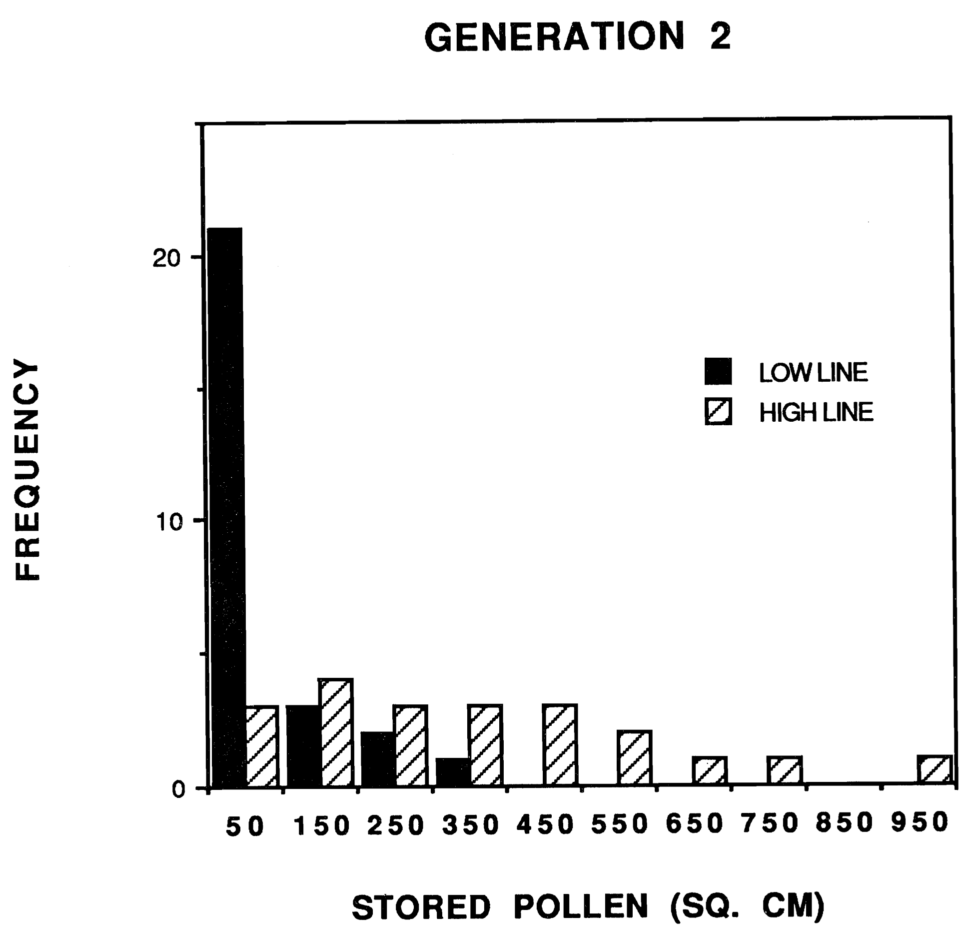

Figure 4. Frequency distribution of generation 2 selected low and high line colonies for area of stored pollen.

Superior performing parent colonies were again selected and the appropriate crosses were made constituting generation 3. Approximately 8 colonies of each subline are currently under evaluation in the University of California Arboretum. Preliminary analyses of the ratio of pollen to nonpollen collectors show that we now have a 40% greater ratio in the high line. High colonies have more than 5 times the stored pollen.

We tested colonies in alfalfa pollination that were derived from naturally mated queens from the generation 3 high and low strains. Colonies with high strain queens had significantly more pollen stored in combs and significantly more brood after 6 weeks of use for pollination (46 and 112 in^2 of pollen and 1.9 and 2.4 frames of brood for high and low strains, respectively). These results suggest that the high strain colonies may provide better pollination for alfalfa and reduce the problem of deficient brood rearing associated with colonies that are maintained in alfalfa fields for extended periods of time.

The results of this selection program suggest the following:

1. Directional selection has been occuring for low pollen collecting in California's commercial bee populations. This is demonstrated by the skewed distribution shown in figure 2 of the commercial colonies tested and by the different shaped curves shown for the frequency distributions of high and low line colonies for selected generations 1 and 2 (Figs. 3 and 4). This has probably been a consequence of selection by queen producers for colonies that have heavier weights (have more stored honey).

2. Nectar and pollen collecting are traits that covary inversely. Selection for increased pollen reserves results in decreased nectar collecting and selection for decreased stored pollen results in increased nectar collecting. This is demonstrated by the progressive increase in the difference in the ratio of pollen/nonpollen collecting bees between the high and low line colonies, even though there remain to be no differences in colony strengths or in the total numbers of foragers.

3. Selection for increased pollen stores can increase the numbers of pollen foragers in colonies. This is important because pollen stores are relatively easy to measure and can easily be incorporated into a commercial breeding program. As a consequence, colonies with high line queens should be at least 30 - 40 percent better pollinating units than standard commercial colonies. I base this claim on the assumption that the low line colonies in these tests are similar to commercial colonies because of deliberate or inadvertant directional selection for low pollen collecting in commercial populations. This may not be true fro those cases where nectar collectors are equally effective pollinators. We plan to test the high and low lines against commercial stocks in alfalfa and almonds in 1991-1992.

4. Selection for low pollen collecting may produce superior honey producing colonies. This conclusion is suggested from the result that low pollen collecting colonies have a greater proportion of nectar collectors. We plan to test this.

Commercial Test of Pollen Hoarding Stock

I am discussing this separately from the discussion of research on the development of the high and low pollen hoarding stocks because it specifically addresses objective 6. This year we initiated a commercial test of the high pollen hoarding stocks for alfalfa pollination and will continue the test next year with almonds and again with alfalfa. What is unique about this test is that it has been done in collaboration with a pollination broker, seed producer, queen producer, and a beekeeper that sells pollination services. The use of selected stocks must be economically sound before they are of any use to the industry. Therefore, we have directed this project to developing a new integration of university stock improvement programs with the crop production and bee industries. The objective is to get growers to share the additional costs of selected bee stocks with the beekeepers. In return growers get better managed hives with superior stocks to pollinate their fields. (After Africanized bees are in California, growers will get European colonies.) My breeding program produced the stocks and provided breeder queens to queen producers. Queen producers raised queens that were paid for by growers. The pollination broker worked with the beekeepers to insure that the queens were placed in hives and moved into the appropriate fields and orchards. My staff will evaluate the pollination activities of the colonies in the fields and orchards. Our goal is for everyone to make money, if not, the program will fail.

Breeding for Decreased Defensive Behavior

In December 1991 we established a research laboratory in Ixtapan de la Sal, Mexico in collaboration with Mr. Guillermo Garcia of Vita Real. Mr. Garcia graciously donated laboratory space for us in his hacienda. My graduate student, Mr. Ernesto Guzman-Novoa is currently working there with three goals: 1. to determine the relationship between the degree of Africanization of a colony and defensive behavior, 2. to determine the amount of backcrossing of Africanized to European stocks of bees that is necessary to result in defensively manageable bees, and 3. to monitor the progressive Africanization of Mr. Garcia's queen rearing operation as it occurs. These goals support program objectives 1, 2, 3, and 7.

To obtain goal 1, we have now completed evaluating colonies for defensive behavior that contain different mixtures of Africanized and European workers. This study was conducted at our Mexico field laboratory site in Ixtapan de la Sal. Pure European queens were instrumentally inseminated with semen from varying proportions (0, 0.25, 0.50, and 1.00) of African drones (I will call drones from Africanized colonies used in this study "African" rather than Africanized to distinguish them from the resultant hybrid workers derived from European queen mothers that I will call Africanized). Resultant colonies then contained varying degrees of Africanization from 0 (only European drones used) to 50%(only African drones used). Additional "African" colonies were used for comparison and are referred to as 100% Africanized. Colonies were tested for stinging behavior by systematically waving a black leather patch in front of the entrance and waiting for the first sting. No additional stimuli were provided to induce stinging behavior. The elapse time was recorded until the first sting was received and the patch was presented for 60 additional seconds. The number of stings received in the 60 seconds following the first sting was then determined.

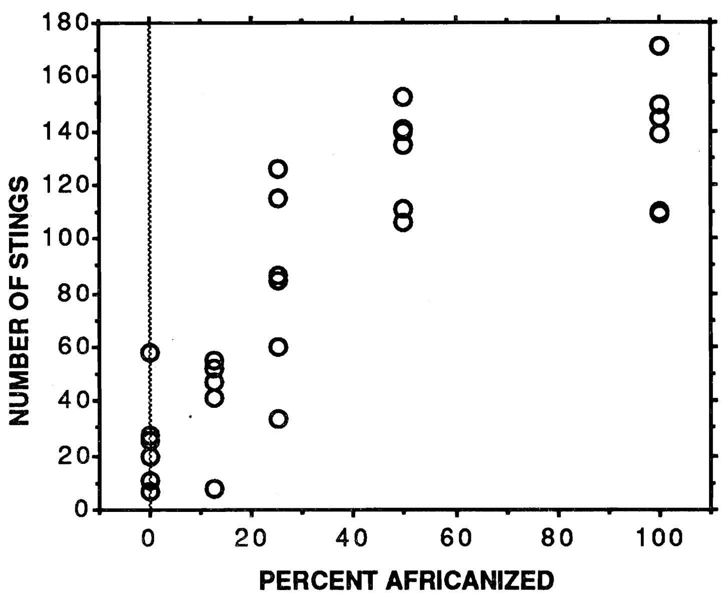

Figure 5.

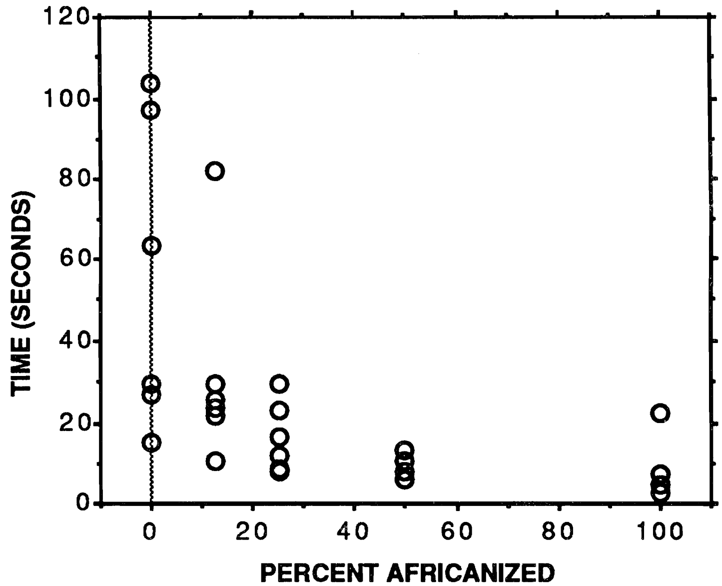

Figure 6.

The results of this study suggest that the extreme defensive behavior of Africanized bees is a dominant trait of individual workers (see Figs. 5 and 6). This is demonstrated by comparing the 50% and 100% Africanized colonies. The colonies consisting of all hybrid workers (50% Africanized) gave the same level of defensive response as the 100% Africanized colonies. Colonies that consisted of varying mixtures of Africanized and pure European workers gave defensive responses that were proportional to the proportion of hybrid workers in the colonies.

For goal 2, 12 queens were inseminated with semen from single drones to constitute generation 1. Colonies derived from these queens are currently being managed and will be tested for defensive behavior then daughters will be raised from them and backcrossed to drones from European queens. We will do this for several generations and, from this study, determine the efficacy of attempting to change the behavioral composition of Africanized feral and/or commercial bee populations by flooding areas with European drones resulting in progressive "Europeanization" through successive backcrossing.

To achieve goal 3, Mr. Guzman-Novoa is taking periodic samples of workers produced in colonies that are used for queen production. These colonies are located in apiaries that vary in locations from about 1,000 to 2,500 meters in elevation over about 80 horizontal kilometers. These samples will be analyzed for morphometric, allozyme, and DNA indicators of Africanization. From these, we hope to be able to document the process of Africanization and, perhaps, offer advice to Mr. Garcia that will aid him in maintaining his stocks relatively free from Africanization. This information should be valuable for providing information about what to expect in California queen rearing areas and for formulating a program to minimize Africanization in those areas.

So far, Mr. Garcia's apiaries are relatively free of Africanization. However, this is not encouraging because Africanized swarms only started occuring in his area in the fall of 1990 and they are now common. Despite the fact that he requeens all 4000 of his colonies at least twice a year, he already has some colonies that are unmanageable and appear to be Africanized. We have not yet been able to analyze the samples that we've taken and it is likely that we will not be able to "keep up" with the process of Africanization and help Mr. Garcia.

Characterize California Feral Honey Bees

This project supports objectives 1, 2, 3, and 4. California will probably be a mosaic of Africanization because of its length, north-south orientation, and topography. It is likely that southern and coastal areas will be Africanized but northern and mountainous areas will not. I have no idea what desert areas will be like. Therefore, it is important to study the feral populations of California so that we have base line data against which to compare those populations after Africanization occurs. If we can identify areas that have remained isolated from the Africanization process, then we can use them as refugia for European stock production for commercial beekeeping. In addition, a study of the feral population will yield expectations for the potential impact of the commercial bees on the feral population. If we find that the feral population is relatively isolated reproductively from commercial bees (which could occur as a consequence of commercial beekeeping practices), then we can expect that our European commercial colonies will have little effect on changing the characteristics of the Africanized, feral population.

Last spring and summer we sent out over 1000 letters to organizations and government agencies throughout California asking for help locating feral colonies of honey bees. We had numerous responses and so far have been able to collect samples from more than 150 colonies distributed from San Diego to Eureka; we plan to collect more samples this summer. We have started morphometric analyses of our most southern samples using the FABIS method. We started with samples in Imperial and San Diego counties because this is likely to be the first area of California to become Africanized. We have found that feral colonies in Imperial county frequently are classified as highly Africanized with FABIS I. This is not surprising because Howell Daly, U. C. Berkeley, also studied the morphometric relationships of feral colonies of California and found that southern California, desert-type bees were misidentified or were unidentifiable by his morphometric analyses, even after he adjusted his data base to include the feral population.

Therefore, we are concentrating our efforts in the southern California desert regions to establish a data base of DNA markers that will reduce the likelihood of misidentification of European bees as Africanized.

The feral bee study is an enormous undertaking requiring careful planning and organization. Processing and analyzing the data will take many man hours of labor and will require the development of new DNA technology (discussed below). When it is completed, as outlined above, it will only represent the baseline prior to Africanization. It needs to be an ongoing project for at least five years following the expansion of Africanized bees into California. The Alfred P. Sloan Foundation is co-supporting this project for two years by sponsoring a Fellowship in Molecular Evolution in my laboratory at about $40,000 per year.

Development of DNA Markers to Detect Africanized Bees

This project supports objectives 1, 3, and 4. The use of DNA markers is important for characterizing the feral population of California, determining the impact of commercial bees on the feral population, and augmenting the current morphometric method of determination. Misidentification of European bees as Africanized can have severe economic, social, and political consequences. It is important that we have the best possible tools at our disposal to avoid unnecessary hardships to California agriculture resulting from unnecessary regulation of the bee industry. (Also, we should try to minimize public alarm.) Therefore, my laboratory has been developing new, cutting edge DNA technology directed toward fast, reliable diagnosis of Africanized bees.

To date, we have established a restriction fragment library of honey bee DNA consisting of more than 500 clones. Preliminary analyses showed that California bees are polymorphic for some of these fragments, suggesting that they may be useful as markers for distinguishing degrees of Africanization.

However, the use of restriction fragment polymorphisms is laborious and slow. Therefore, we have developed new technology using DNA amplification by polymerase chain reaction. Reactions are "primed" using randomly constructed 10 base pair oligonucleotides (called RAPDs). So far we have screened 191 different primers, 160 of which have demonstrated polymorphisms that may be useful as population genetic markers. Some of these have been screened against Africanized bees from Mexico and look promising for distinguishing Africanized bees from California's, European bees. Thirty eight primers have been studied in detail and yield an average of 1.6 polymorphisms per primer. We have studied the mechanism of inheritance of two polymorphisms and found them to be Mendelian. Two markers were dominant and one was codominant. Single individuals yield sufficient quantities of DNA for literally thousands of analyses. Alcohol and dried specimens may also be analyzed. A single technician can process 50 - 100 samples per day using our current technology, however, we think that quantity can be increased significantly.

We have had specific primers made for mitochondrial DNA analyses. Primers were made for a DNA sequence that shows variability between African and European bees. Africanized bees usually carry the African marker. We have not yet perfected the technology to do the mitochodrial DNA analyses because we have concentrated out efforts on nuclear DNA.

Development of Instrumental Insemination Technology

A small amount of funds from this project aided the development of a new instrumental insemination apparatus by Dr. Harry Laidlaw. We encouraged the development, built a test instrument, and trained a graduate student to use it. The new instrument costs about $60.00 to construct from parts that are available through standard parts catalogs (other commercially available instruments cost over $700.00). The rationale for developing this instrument was to make it inexpensive enough that potential bee breeders will be encouraged to build it and learn to use it. One instrument is currently in use at our Mexico laboratory. This work supports objective 5.

Long-term Storage of Queens

This work has been done by Dr. Norman E. Gary with minimal funds provided by this project and addresses objectives 1, 2, 5, and 6. Better control of breeding stocks can be acheived if we can develop technology to store large numbers of mated queens for long periods of time. Current methods require that queens be stored in strong nursery colonies with free-flying workers, and are only marginally successful. However, what is needed is the ability to store queens for months, or even years in controlled, confined environments. This would not only serve to preserve and isolate desirable stocks from Africanization, but could also serve as method of quarantine for importing stocks into California.

Random aged and young (< 7 days old) workers were used to maintain mated queens confined in individual cages in incubators held at either 31 or 34 degrees Celsius and 55% relative humidity. Ninty-three percent of queens held at 31 degrees with young workers survived three months. This was the best result and is offers great promising for further development of queen storage technology.