Project Report 1995

TITLE: **Africanized Honey Bee Research**

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Objectives

Objective 1: develop methods to maintain and produce commercial honey bee stocks that are free from the influence of Africanization

Objective 2: develop programs that will allow selective breeding and stock improvement of resident honey bee populations following Africanization

Objective 3: determine the efficacy of and develop methods for improving the genetic composition of feral honey bee populations following Africanization

Objective 4: develop improved methods for analyzing mitochondrial and nuclear DNA in order to determine the range and degree of Africanization throughout California

Objective 5: develop better breeding techniques including instrumental insemination

Objective 6: develop new apicultural practices for commercial beekeeping

Project Overview

This project was initiated in 1989 with the specific objective to study the potential impact of Africanized Honey Bees on California agriculture and to develop methods of honey bee management that could be used to maintain commercially acceptable colonies following Africanization. Over the past 6 years, we have directed our research toward those objectives in the following ways:

1. We have selected strains of bees that demonstrate a 1.8 fold increase in pollen foraging effort when compared with commercial bees. These bees also store more pollen, maintain stronger colonies over the winter, and build up faster before the almond bloom, thereby providing larger pollen forager populations per colony than colonies of unselected commercial bees. High pollen foraging strains have also been shown to have more brood and more stored pollen when used for alfalfa pollination.

2. We studied the genetic basis of defensive behavior in Mexico and found that the genes responsible for the high defensive behavior of Africanized bees are dominant in inheritance

over the less defensive European bees. This leads to very defensive colonies with even low levels of Africanization.

3. We tested the official United States Department of Agriculture method of identifying bees and found that it failed to detect colonies with intermediate levels of Africanization. These same colonies were extremely defensive, thereby demonstrating that those identification procedures are not suitable for the regulation of honey bees in California.

4. We developed a new, mitochondrial DNA test for Africanization that has been implemented by the California Department of Food and Agriculture to monitor the natural spread of feral Africanized bees in California.

5. We have tested methods of introducing queens into colonies and found that the success rate of beekeepers is much lower than they had previously assumed. Our studies resulted in the development by the bee industry of a new design for queen introduction cages and an awareness that more frequent requeening may be necessary to control against the effects of Africanization of commercial colonies.

6. We studied the composition of the feral honey bee population of California and found that it was reduced by about 85% following the spread of Varroa mites between 1991 and 1994. This resulted in a severe loss of potential competition of feral European bees and may increase the rate of spread and the eventual range of Africanized bees in California.

7. We found that populations of Varroa build up much faster in colonies in California than was previously believed. This results from our longer brood rearing season and the robbing behavior of colonies during periods of resource dearth. As a consequence, beekeepers must treat their hives at least twice each year in order to keep their colonies alive.

8. Africanized bees were first found in California in October of 1994 and have not, yet, spread very rapidly. It is possible that the Varroa mites are also responsible for their reduced rate of spread, and may eventually stop their spread.

9. In 1992 we initiated a breeding program in Mexico to determine if we could raise commercially acceptable bees in an Africanized area. Each generation we have measured honey production and defensive behavior of colonies derived from queens produced by standard queen rearing methods employed in California. So far, we have managed to keep producing manageable, productive colonies.

10. We conducted a survey in Mexico to determine the impact of Africanized honey bees on commercial beekeeping. We found that Africanized honey bees increased operational costs about 40%.

Research Results for 1995

Africanized Bees in California

Africanized honey bees were first detected in California last fall when they suddenly appeared at a prison near Blythe. Since then, more colonies have been detected, near Blythe and throughout the Imperial Valley. Each of these colonies was determined to be Africanized on the basis of the USDA-ID morphometric method that analyzes 21 different size and body part characteristics. In addition to the standard USDA-ID method, the California Department of Food and Agriculture pest diagnostic lab analyzed the mitochondrial DNA of these bees using a technique developed in my laboratory by Dr. Paul Ebert. They found that all of these colonies had African-type mitochondria. The mitochondria in honey bees (and humans) is inherited only from the mother, therefore, these results demonstrate that the Africanized bees arriving in California are part of a very long maternal line that extends relatively unchanged back to the original introduction of African bees into Brazil in 1956.

Two years ago Africanized bees were found on the southern and eastern borders of California. I believed at that time that their entry into California was inevitable and had probably already occurred. My hypothesis was that they would increase in density in that area until they were numerous enough to be captured in a trap, or cause some kind of problem in an inhabited area. The repeated finds of Africanized bees in the Imperial Valley this spring stimulated me to sample bees from that region in order to determine their distribution and relative abundance.

The standard USDA-ID method requires sampling 10 bees from a single colony. Feral colonies are not easily found unless they fly into traps or are a nuisance to someone, so the morphometric method is not good to use as a random survey tool. Mitochondrial DNA analyses, however, can be made on single bees. Therefore, an area survey is possible by simply catching bees on flowers, then determining their mitochondrial type. If Africanized bees are abundant in an area, relative to European bees, then there should be an abundance of African mitochondrial types in the sample.

April 13-15, I collected 75 bees from 31 collection sites located in the Imperial Valley and near Blythe, California. We tried to make collections in areas where bees were likely to be feral, although there is no way to tell where the bees came from. Mitochondrial DNA diagnostics were run on these bees and we did not find any mitochondrial types that are indicative of Africanized honey bees. I interpret these results to suggest that Africanized honey bees are not yet abundant in these areas. My original hypothesis was probably wrong. Africanized bees have not been in California for two years; it is likely that we are truly seeing the very early stages of the "invasion" of Africanized bees. The current methods of sampling used by the California Department of Food and Agriculture -- trap lines and reports from concerned citizens -- are better than random sampling methods for early detection of rare Africanized colonies. However, the method of randomly sampling bees and checking their mitochondrial DNA will be the best indicator of the extent of "Africanization" in an area.

Why it has taken them so long to move into California, and why they are spreading so slowly remains a mystery. Genetic mixing with commercial bees does not seem to be the answer because the colonies that have been detected have been highly Africanized both in morphometric characters and mitochondrial DNA. Only time will tell to what extent they will spread and how abundant they will become, but for now they have not made a detectable impact, even in the Imperial Valley.

I plan to conduct another survey during the spring of 1996. This survey will be more extensive.

Breeding Bees in Africanized Areas

We continue our breeding program in Ixtapan de la Sal, Mexico. This will be our final season of collecting extensive honey production and defensive behavior data. A preliminary analysis of our data show that we have successfully reduced the defensive behavior of our commercial bees that are produced using normal queen rearing and mating methods in a highly Africanized area. At the same time, we have decreased the degree of Africanization of our commercial bees as indicated by an increase in the size of our bees and by a decrease in the prevalence of African mitochondria. In addition, our colony average honey production has stayed fairly constant during the 5 years of our program while that of other beekeepers in the same area have declined precipitously.

I think this program has been very successful and is encouraging for the future of bee breeding and queen production in Africanized areas of California. Our next objective is to streamline the breeding methods so that they are more easily performed and adapted by California beekeepers.

Breeding High Pollen Collecting Bees

We are now in our 5th year of this program. We have selected bees that show a strong preference for collecting pollen and have characterized the stocks with respect to their foraging behavior. Colonies of high strain bees have 1.8 times more pollen foraging activity compared with commercial bees. This should result in 1.8 times more pollinating activity per colony. We have also demonstrated that colonies composed of high pollen strain workers build up faster in the late winter and early spring. Although we have not been able to get beekeepers interested in using these stocks, we will continue to work with beekeepers to evaluate their commercial values.

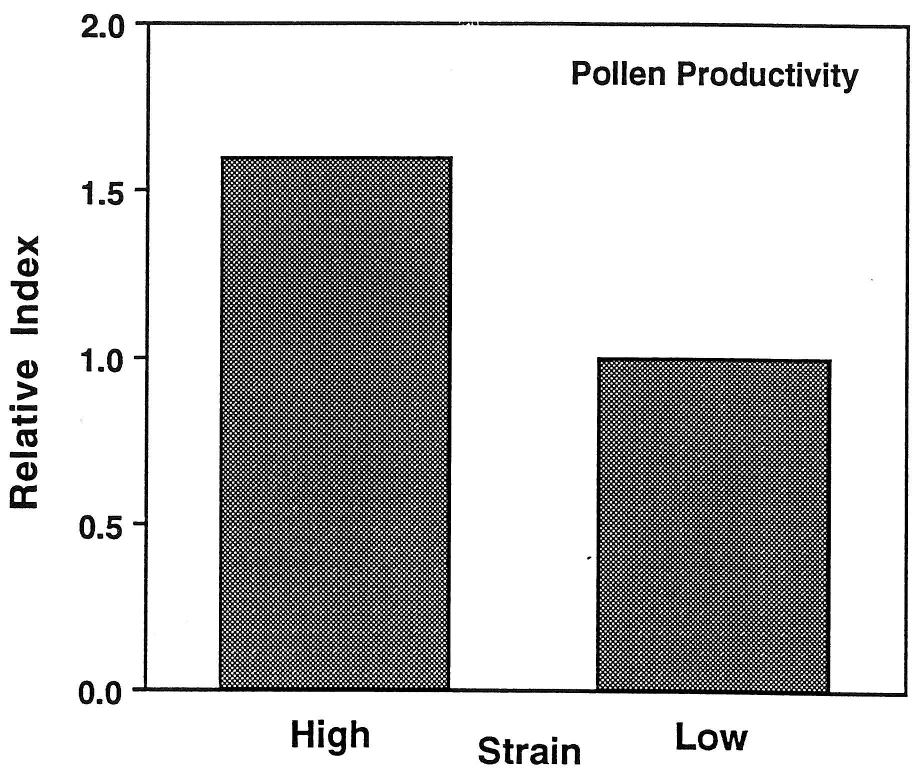

Figure 1. Relative pollen collecting productivity of high and low strain workers when raised in common, unselected colonies, and evaluated in controlled flight cages. High strain foragers were 1.6 times more productive.

We are now beginning to understand the behavioral mechanisms that lead to the observed differences in pollen and nectar foraging of our selected strains. Our selection program has resulted in both high and low pollen foraging strains. The low strain colonies store very little surplus pollen. In one study, we raised high and low strain workers together in commercial nucleus colonies that were placed inside enclosed screen cages. We offered pollen and nectar at feeders within the cages and observed the foraging behavior of the high and low strain workers. We recorded the numbers of times individuals were observed collecting nectar or pollen, and collected some high and low strain workers as returned from their foraging flights and weighed their nectar and pollen loads. High strain workers were more likely to collect pollen, made more pollen foraging trips per day, and collected larger pollen loads than low strain workers. From these data we calculated their relative per day productivity for pollen (Fig. 1) and found that high strain foragers were more productive pollen foragers even when raised in a foreign colony. In a similar study, we found that unselected commercial bees were intermediate in pollen collecting productivity.

High strain pollen foragers are also more likely to recruit other pollen foragers. Honey bees have a communication system that recruits new foragers to pollen and nectar sources. A foraging bee returns to the hive and performs a "dance" on the comb that "excites" other potential foragers and results in an increase in foraging activity at the dancer's foraging location. When a high strain forager returns to the hive with a load of pollen she is likely to perform a more vigorous recruitment dance and get more potential recruits excited about pollen foraging (Fig. 2). Therefore, high strain workers are individually biased to forage for pollen, make more foraging trips, collect larger loads, and recruit more vigorously, resulting in significantly more pollen foraging and presumably more pollination activity.

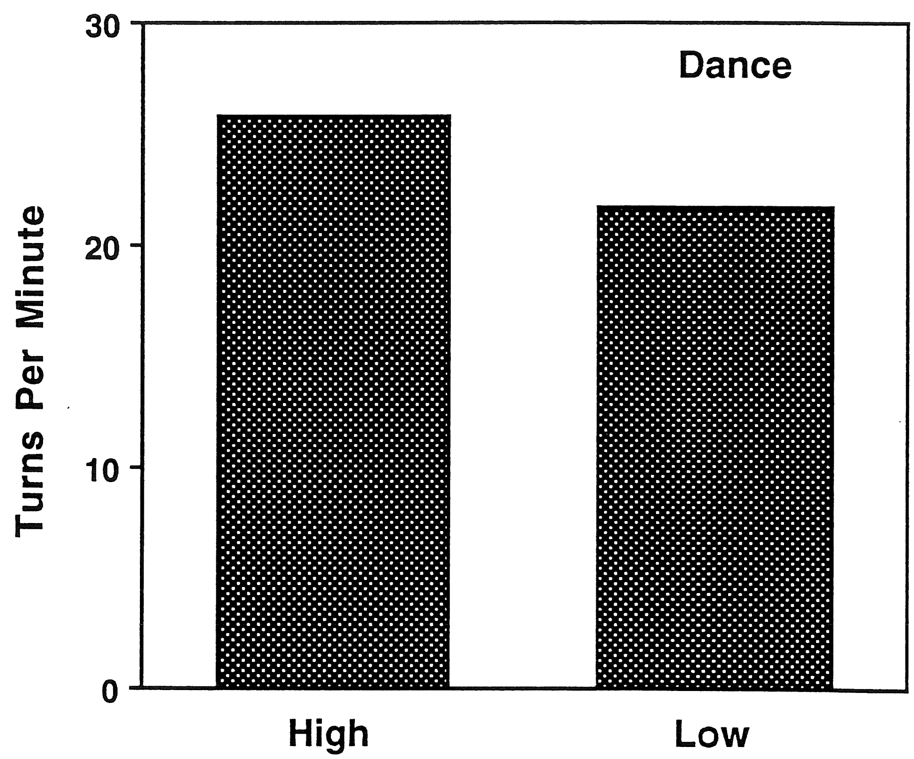

Figure 2. The difference in the vigor of dances of pollen foragers of the high and low strains. The number of turns per minute is an indicator of how vigorously a forager is recruiting new foragers to her resource and correlates with the number of new recruits that eventually arrive.

We have also characterized some of the underlying genetic basis of this behavior. Two major genes (we have named these *pln1* and *pln2*) are influencing the decisions of honey bee workers to forage for pollen and nectar. In a colony, there are three classes of workers: those that collect only nectar, those that collect both nectar and pollen (generalists), and those that collect only pollen (Fig. 3).

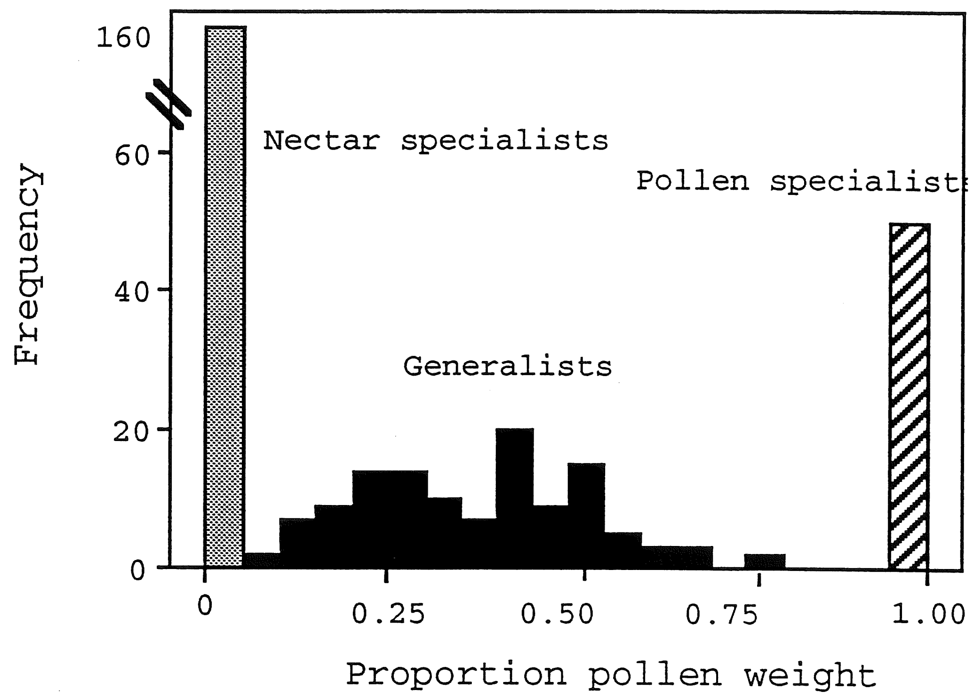

Figure 3. The number of individuals (frequency) belonging to three classes of foragers in a the colony discussed below. Returning foragers were collected at the entrance of a colony, their nectar and pollen load weights determined. Workers were segregated into three classes on the basis of the proportion of their total load weight that was pollen, then their DNA was analyzed to determine how many high pollen hoarding genes they inherited from their parents.

The number of high and low pollen hoarding genes inherited from their mothers and fathers strongly influences which class they will join. We performed specific crosses of high and low strain queens and drones to produce a colony where workers had different numbers of high and low pollen foraging genes at those two loci. We then collected foragers as they returned from their foraging flights, recorded whether they carried pollen, nectar, or both, then analyzed their DNA to determine which genes they inherited. We found that individuals that inherited more high pollen foraging genes were more likely to collect pollen (Fig. 4).

Pollen Foraging and the Hive Environment

The hive environment, as well as the genetic composition of workers, is important for determining the number of pollen foragers in a colony. Our studies have shown that colonies with more workers have more foragers and, consequently, more pollen foragers. In addition, the presence of more young brood stimulates more pollen foraging while the presence of more stored pollen reduces pollen foraging. The presence of empty combs above the brood nest stimulates more foraging for both pollen and nectar while empty combs below the brood nest have no effect on foraging.

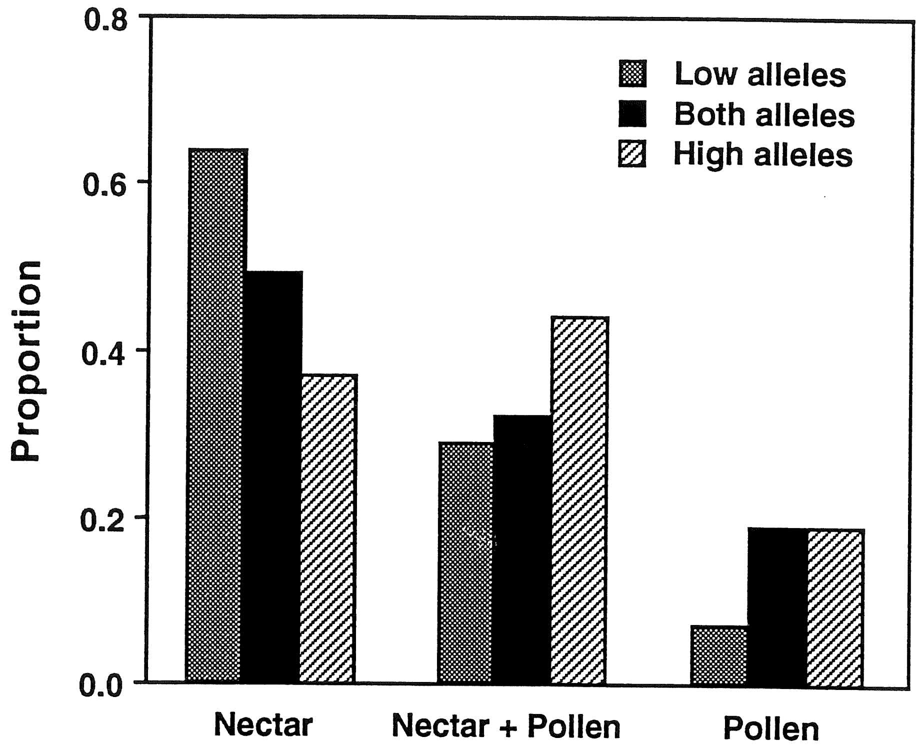

Figure 4. The proportion of individuals sampled that collected only nectar, both pollen and nectar, or only pollen that inherited 0 (Low alleles), 1 (Both alleles), or 2 (High alleles) high pollen foraging genes (alleles) from their mother. The father of these workers had only high pollen foraging genes. The queen mother was a hybrid having one high and one low allele at each of the two loci, *pln1* and *pln2*.

Varroa Mites and Africanized Bees

The parasitic mite, *Varroa jacobsoni*, is currently the single most important problem for California beekeepers and is and the most significant threat to the supply of bees used for pollination. Reports from South America have suggested that Africanized bees are resistant to Varroa. If this is true, then we may be able to study the mechanisms of resistance in Africanized bees and use this information to develop breeding programs for resistance in European bees. We conducted studies in Mexico and found that when given a choice, female Varroa mites prefer to feed on European adult and larval workers (Figs. 5 and 6).

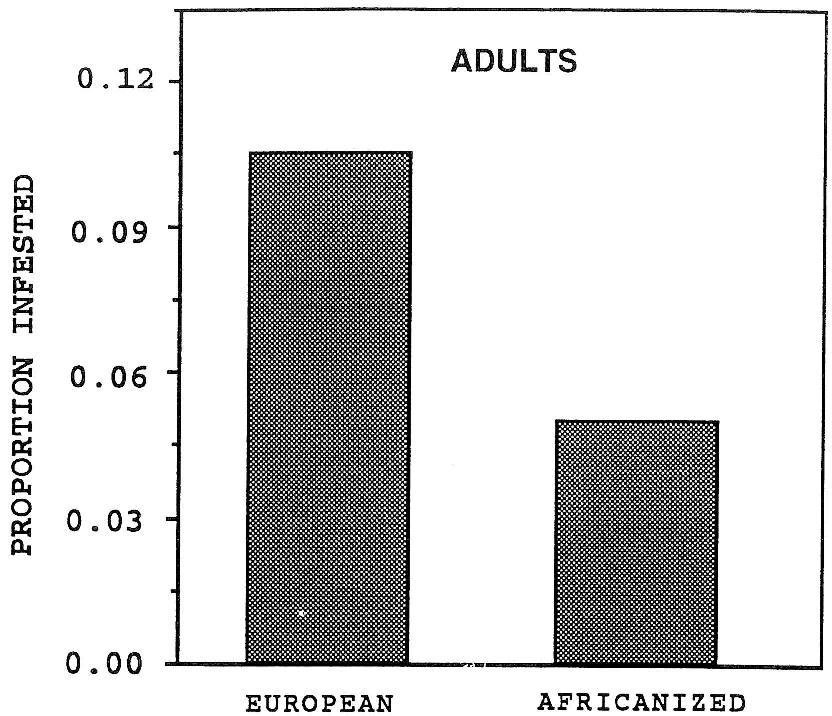

Figure 5. Proportion of European and Africanized adult honey bees with Varroa. Adult workers were placed into a colony infested with Varroa. Two weeks later, they were recovered from the colonies and inspected for the presence of Varroa parasitic mites.

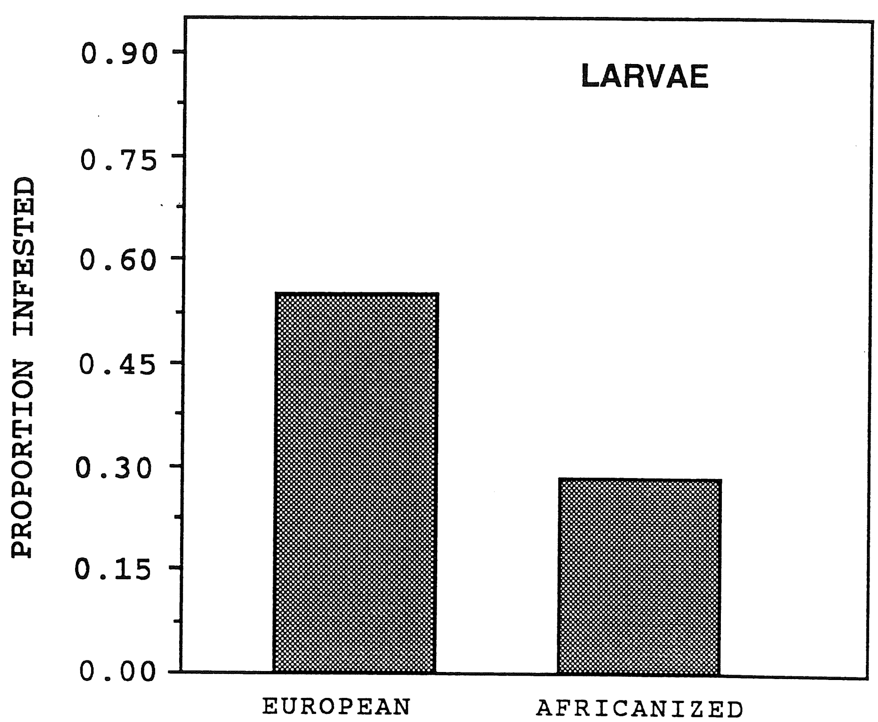

Figure 6. Proportion of larvae derived from European and Africanized honey bee colonies that were infested with Varroa. Brood from European and Africanized colonies were placed into a colony that was infested with Varroa. Fifteen days later, the combs containing brood were removed and each cell was inspected for the presence of Varroa on the brood.

Recommendations

Geographical Range of Africanized Bees

Annual surveys should be taken to determine the extent of the spread of Africanized bees throughout California. These surveys should be random and should be done using the mitochondrial DNA diagnostic technique that we developed. Through these surveys beekeepers and growers will be aware of the Africanized honey bee risk factors within any given area and can make informed management decisions.

Management for Pollination

Our studies suggest that greater pollination activity can be achieved by colonies that have been selected for high pollen hoarding behavior. The genetic basis of this behavior suggests that it is a trait that can be easily selected by beekeepers. In addition, maximum pollen foraging activity will be achieved if colonies are manipulated to have larger forager populations, more brood, less stored pollen, and ample empty comb space above the broodnest.

Research for Varroa

Research should be conducted to determine the mechanisms of Varroa resistance that are evident in Africanized bees.