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### **1992 ANNUAL REPORT FOR ALMOND RESEARCH BOARD**

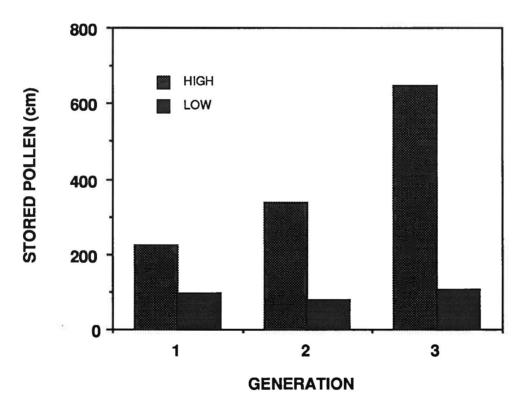
## **AFRICANIZED HONEY BEE**

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Our research over the past year has been directed toward our six objectives listed above. In this section, I will briefly review research associated these objectives and discuss how they relate to commercial beekeeping and pollination. In the next section I will discuss our recent experimental results and observations relating to each objective.

We have continued selecting for high pollen hoarding strains of bees that may be used in commercial pollination. This selection program was designed to demonstrate methods of honey bee breeding that could be used by queen producers in California following the arrival of Africanized bees, about 2 or 3 years from now. It is important that we establish the infrastructure for producing and distributing good, European stocks of honey bees in order to maintain a queen rearing industry in this State. Selection for pollen hoarding was initiated because pollen hoarding is directly associated with pollen collecting and pollination. It is likely that our current problems with mite parasites of bees, and the inevitable Africanization of California honey bees, will lead to short supplies of bees in the future. High pollen hoarding strains of honey bees may provide more pollination service per hive than the commercial strains currently used. We have also been developing and testing honey bee identification methods that may be used for monitoring the spread of Africanized bees. It will become important for beekeepers and growers to know what areas of California are Africanized in order to make informed management decisions. These methods will also be useful for aiding queen producers in selecting their stocks and assessing how their management practices affect the degree to which their bees become Africanized. A component of this objective is to characterize the genetic composition of our current feral population and assess the degree to which it is genetically integrated with commercial bees.

Two years ago we initiated research in Mexico to better understand the genetic basis of the extreme defensiveness of Africanized bees. We reported our results at last year's meeting. We have continued our work in Mexico. We have been monitoring the bees of a commercial beekeeper, giving him management advice to try and prevent his bees from becoming Africanized. This year we set up a new selective breeding program, using his bees, in an area with Africanized feral bees. We started with European bees and are attempting to select gentle, productive bees while they become Africanized. We are using selection methods that are simple and likely to be implemented by California beekeepers.

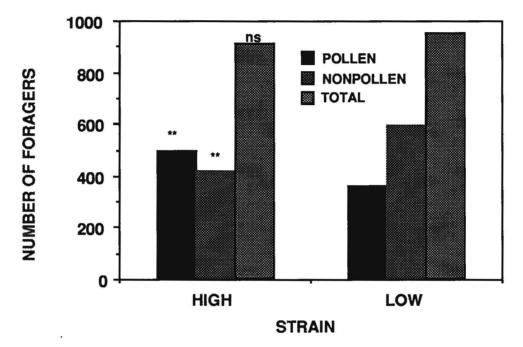


# **RESPONSE TO SELECTION**

Figure 1. Results of the first three generations of two-way selection. We tested 24, 22, and 27 high and 27, 27, and 31 low strain colonies in generations 1 - 3, respectively. Selection methods were identical for the first three generations. Experimental methods varied for generations 4 and 5, therefore, results of those generations are shown separately.

### **Selection for Pollen Hoarding**

We have now completed 5 generations of two-way selection for high and low pollen hoarding (see Figs. 1, 3 and 5). We continue to increase the differences between our high and low strains with respect to the amounts of pollen stored in the comb. The observed increases are primarily due to increases in the high strain. Low strain average pollen stores have not changed since generation 1. We have repeatedly demonstrated that increased pollen stores are a consequence of increased pollen collecting by workers within colonies (Fig. 2).



**GENERATION 3** 

Figure 2. Mean values of foraging activities of colonies from generation 3. Entrances of 27 high and 31 low strain colonies were observed for 12 minutes. All returning foragers were observed and counted as carrying a load of pollen or not carrying a load of pollen (nonpollen). Our results demonstrate that there were no differences in the numbers of foragers between strains but high strain colonies had a significantly greater proportion of foragers carrying loads of pollen.

We tested colonies of generation 4 in almond orchards in 1992. We compared the performance of 20 high strain colonies with 16 colonies of our low strain and 15 unselected commercial colonies. The commercial colonies performed intermediate to our high and low strains, as expected. The high strain colonies had 1.3 times higher ratio of pollen to nonpollen collectors compared with commercial colonies. We collected bees returning with pollen, weighed their loads, and found that the average load of a worker from a high colony was 1.4 times greater than a commercial bee. From these values we estimate that high colonies were collecting about 1.8 times more pollen than commercial colonies.

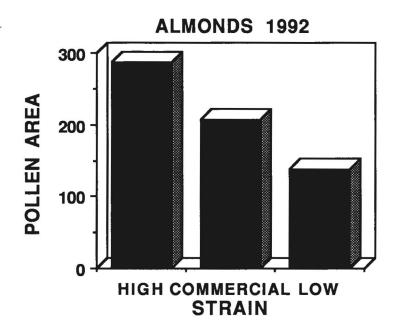


Figure 3. The average amount of pollen stored in high, low, and commercial strains of bees maintained in almond orchards. High and low strain colonies were of generation 4.

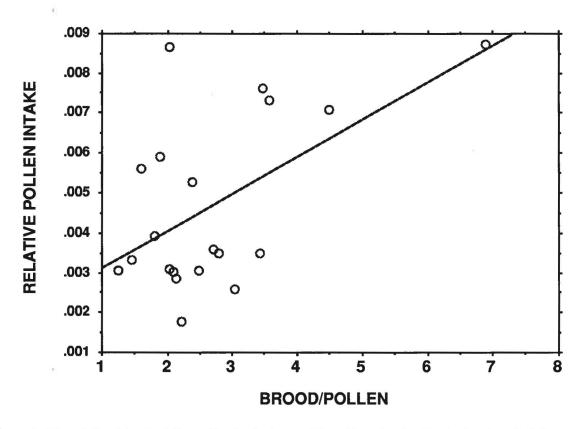
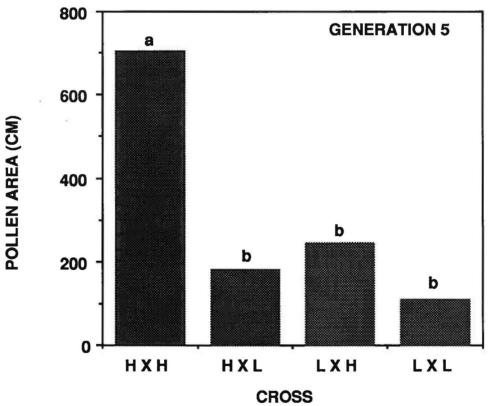


Figure 4. The relationship of relative pollen intake to quantities of brood and pollen in the hive. Relative pollen intake is the product of the proportion of foragers collecting pollen and the average weight of a pollen load in that colony. Intake increases significantly as the ratio of brood:pollen increases.

Continued selection for high quantities of stored pollen has resulted in decreased brood production in our high strains, under some test conditions. When colonies are maintained in single-storied hives, space becomes restricted and pollen stores reduce the area available for brood rearing. In fact, in generation 5, our high strain colonies had significantly more stored pollen, less brood, and fewer pollen foragers than our low strain. We did not have this problem in generation 4 (the almond orchard test) because we provided two hive bodies for the brood nest and the pollen stores did not reduce the area available for brood rearing. Increased quantities of brood stimulate more pollen collecting while increased quantities of pollen reduce pollen collecting. This relationship of brood area and pollen area stimuli was evident even in the two story hives of our high strain colonies of generation 4 (Fig. 4).

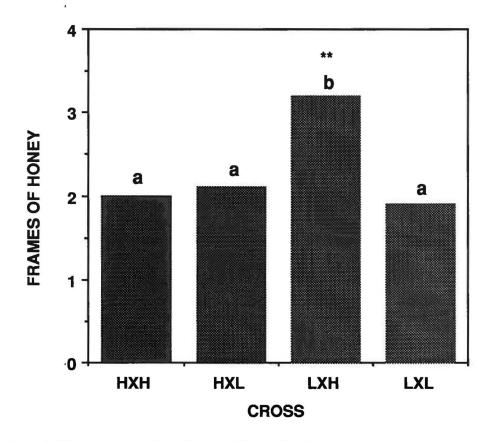
In generation 5, we crossed queens and drones of the high and low strains to determine the mode of inheritance of pollen hoarding behavior. We compared high X high (H X H), low x low (L X L), high X low (H queens X L drones), and low X high (L X H) strain crosses. Our results demonstrate that high pollen hoarding behavior is recessive to low pollen hoarding (Fig. 5). Our hybrid colonies were not intermediate between the high and low strains, but, instead, performed like the lows. This explains the ease and rapidity with which we were able to select our high pollen strains. It is easier to select and fix a trait that is recessive than one that shows dominant inheritance.



# **GENETIC COMPONENTS**

Figure 5. Average area of pollen stored in combs of 5 H X H, 8 L X L, 7 H X L, and 6 L X H colonies of generation 5.

We also measured the amount of honey stored in colonies of generation 5. We found that, like pollen hoarding, honey production is nonadditive in inheritance. In this case it looks like some particular crosses result in hybrid vigor for honey production (see L X H, Fig. 6). We found no phenotypic correlation between honey production and stored pollen.



# HONEY PRODUCTION

Figure 6. The average number of frames of honey for colonies of generation 5.

#### **Conclusions and Recommendations**

Our results demonstrate that the pollinator activities of colonies can be increased by selective breeding. An individual colony of our high strain provides the pollinator activity of 1.8 unselected commercial colonies of equivalent strength and condition. Beekeepers can easily select for increased pollen collecting by selecting for increased stored pollen. Rapid selection response is expected because of the recessive inheritance of high pollen hoarding. Honey production seems to be an independent trait with a different mode of inheritance. Therefore, selection for high pollen collecting should not necessarily result in reduced honey production.

An unexpected outcome of this study was the strong effect of the ratio of brood/stored pollen on the pollen collecting activities of colonies. These results suggest that the quantity of brood should be assessed as well as the population of workers when assessing the pollinating value of a colony. Colonies should also be managed to reduce the effects of pollen stores on the reduction of brood rearing.

### **Identification of Africanized Bees**

We have collected worker samples from more than 200 feral honey bee colonies throughout California. We have analyzed them for a specific protein, malate dehydrogenase, that shows genetic variability. We have compared the frequencies of specific protein variants between different regions of California and between the feral and commercial bees. Our protein data demonstrate that the feral bees of California are not simply escaped commercial bees but exist to a significant extent independent of the genetic influence of commercial bees. Our results support those of Dr. Howell Daly, Department of Entomology, University of California, Berkeley who came to the same conclusion based on analyses of the morphology of feral and commercial bees.

In 1991, we conducted studies of defensive behavior in Ixtapan de la Sal, Mexico. For those studies, we established colonies with varying degrees of Africanization. We did this in two ways: 1. we instrumentally inseminated European queens with semen of European and Africanized drones mixed in specific proportions, and 2. we crossed and progressively backcrossed Africanized queens to European drones. From these crosses we produced colonies that were 100, 50, 25 12.5 and 0 percent Africanized. We tested those colonies for defensive behavior and reported at the Almond Research Meeting last year that the extreme defensive behavior of Africanized bees is dominant in inheritance and that the 50% Africanized (hybrid) colonies were just as defensive as the 100% Africanized colonies.

This year we performed morphometric analyses on samples from those same colonies. Morphometric analyses compare the sizes and relationships of different body parts of bees. These analyses are very good at separating highly Africanized bees from European bees. However, they are most frequently used to monitor the spread of Africanized bees and make determinations during the early stages of Africanization in an area. Morphometric analyses are currently being used for identifying Africanized bees by regulatory agencies within USDA, SARH (Mexico), and the state of Texas. We used the same methods applied by other regulatory agencies to test our bees of known degree of Africanization. Morphometric analyses classified nearly all of our samples as European, including 12 of 16 of our 50% Africanized hybrids. Altogether, it correctly identified as Africanized only 5 of 49 colonies that were Africanized to some degree.

We continue to evaluate our new DNA markers for their usefulness in classifying Africanized bees. We have 10 markers that can be used in combination to discriminate between European bees collected in California and Africanized bees collected in Mexico and Central America. With these 10 markers we correctly classified about 97% of our 155 samples. We are now testing these markers against new "unknown" samples from Texas, Mexico, and California to assess their efficacy. We are also trying to develop more expedient methods of analysis. Currently, one technician in my lab can make determinations on about 10 individuals (colonies) per day.

#### **Conclusions and Recommendations**

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We do not expect the commercial honey bees of California to have a significant impact on feral Africanized bees. Feral bees will probably become irreversibly Africanized in many parts of California making it difficult or impossible to produce European bees for commercial beekeeping in those areas. Morphometric analyses will probably remain the preferred method for determining areas of California that are highly Africanized but will not be sufficient for detecting the lower levels of Africanization that we expect to see in commercial hives. Even low levels of Africanization can dramatically increase defensive behavior of colonies. In our previous studies, reported last year, we showed that colonies that were 50% Africanized were more than 5 times as defensive as colonies of pure Europeans. Therefore, DNA identification markers, such as ours, will be needed if we want to monitor the early stages.

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#### **Mexico Project**

Since 1990, we have been working with the beekeeping establishment of Vita Real in Ixtapan de la Sal, Mexico . When we began, Vita Real was on the edge of the expansion of Africanized bees in Mexico and appeared to be 100% European. The first Africanized bees were detected in Ixtapan in the summer of 1990 and, since that time, the area has become progressively more Africanized. Vita Real maintains about 4000 hives of bees for honey production. They raise their own queens in areas where they attempt to control matings with European drones and regularly replace queens in all their hives. In spite of their efforts, and ours, Vita Real is becoming progressively Africanized. With increased Africanization, they are experiencing more problems associated with management. In particular, they are losing apiary locations due to serious stinging incidents involving humans and livestock.

We have now abandoned our plans to keep Vita Real from becoming Africanized (because we failed) and have implemented a new breeding program. This program is designed to select for low defensiveness and high honey productivity using the bees that Vita Real now has. We are using simple breeding methods that California beekeepers may readily adopt when needed. We are currently testing generation 1.