Project Number: 91-G5

DATE SUBMITTED: JANUARY 1, 1992

TITLE AND CONTRACT NUMBER: AFRICANIZED HONEY BEE #90-0346

PROGRESS REPORT FOR YEAR 1991:

NAME OF INVESTIGATOR: ROBERT E. PAGE, JR.

I. OBJECTIVES

1. Develop methods to maintain and produce commercial honey bee stocks that are free from the influence of Africanization.
2. Develop programs that will allow selective breeding and stock improvement of resident honey bee populations following Africanization.
3. Determine the efficacy of and develop methods for improving the genetic composition of feral honey bee populations following Africanization.
4. Develop improved methods for analyzing mitochondrial and nuclear DNA in order to determine the range and degree of Africanization throughout California.
5. Develop better breeding techniques, including instrumental insemination.
6. Develop new apicultural practices for commercial beekeeping.

II. SUMMARY OF ACCOMPLISHMENTS

We initiated a honey bee breeding program in 1990 that was designed with two objectives. The first objective was to establish a demonstration program of controlled breeding within genetically closed populations. From this program we hoped to solve the basic management problems and show California queen producers how they could raise commercially manageable, preferably European, queens in an Africanized environment. The second objective was to select for traits that are commercially important in order to make such a breeding program economically viable. We have succeeded in both objectives. We have selected four generations for pollen collecting. Colonies from our high pollen collecting lines have about 40% more

pollinators than our control lines. We have implemented a test program using these bees for pollination that involves queen producers, pollination service beekeepers, a pollination contract broker, and alfalfa and almond producers.

In 1990, we established a research laboratory in Mexico. We initiated studies of the genetic basis of defensive behavior and found that the extreme defensiveness of Africanized honey bees (AHB) is a dominant trait. We also found that backcrossing AHB queens to European drones rapidly results in the less defensive, European-like defensive behavior. This gives us hope that we can change the behavior of feral and commercial honey bees in Africanized areas, at least on a local level.

We continue to look for DNA markers that can be used for identification of AHB. We pioneered the use of Polymerase Chain Reaction (PCR) and Random Amplified Polymorphic DNA (RAPD) markers. We have screened more than 600 potential markers without finding any diagnostic trait. However, we have 10 markers that show considerable differences in frequencies between California feral honey bees and Mexican AHB. With these, we should be able to make strong statistical statements about the degree of Africanization of honey bee samples.

BUDGET

Salaries and Benefits	$60,910
Michael Crosland, PGR V	
Charles Dullum, PGR I	
Meredith Humphries, GRA	
Ernesto Guzman-Novoa, GRA	
Supplies and Travel	30,000

Overhead @10%		9,090
	Total	$100,000

Dr. Michael Crosland is only 22.03% on this project. The rest of his salary is provided by a Sloan Foundation Fellowship in Molecular Evolution to study the DNA marker composition of California feral honey bees. This is a two year fellowship from February 1991-January 1993 for $40,250 per year. This study complements the CDFA project.

ACCOMPLISHMENTS FOR CURRENT YEAR

Objectives 1, 2, 5, and 6: Selective Breeding for Increased Pollen Collecting

METHODS: 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.

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).

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. 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.

Superior performing parent colonies were again selected and the appropriate crosses were made constituting generation 3. Approximately 8 colonies of each subline were produced and 27 high and 30 low strain colonies were evaluated in the University of California Arboretum in the summer of 1991.

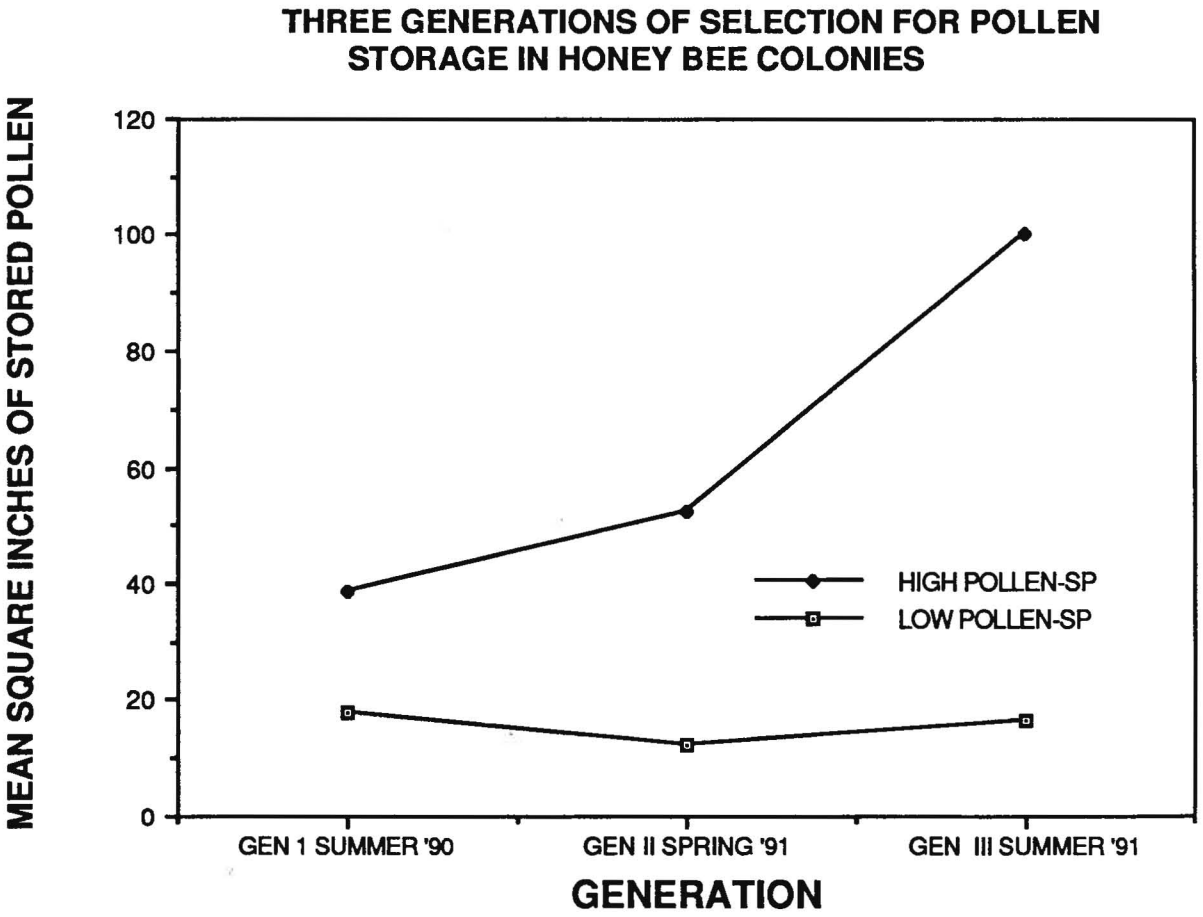

Figure 1. Results of three generations of selection for high and low pollen hoarding.

RESULTS: Selection has continued to separate the high and low strains with respect to quantities of stored pollen. Generation 3 colonies had average pollen stores of 17 and 100 in^2 for the low and high strains, respectively (see Fig. 1).

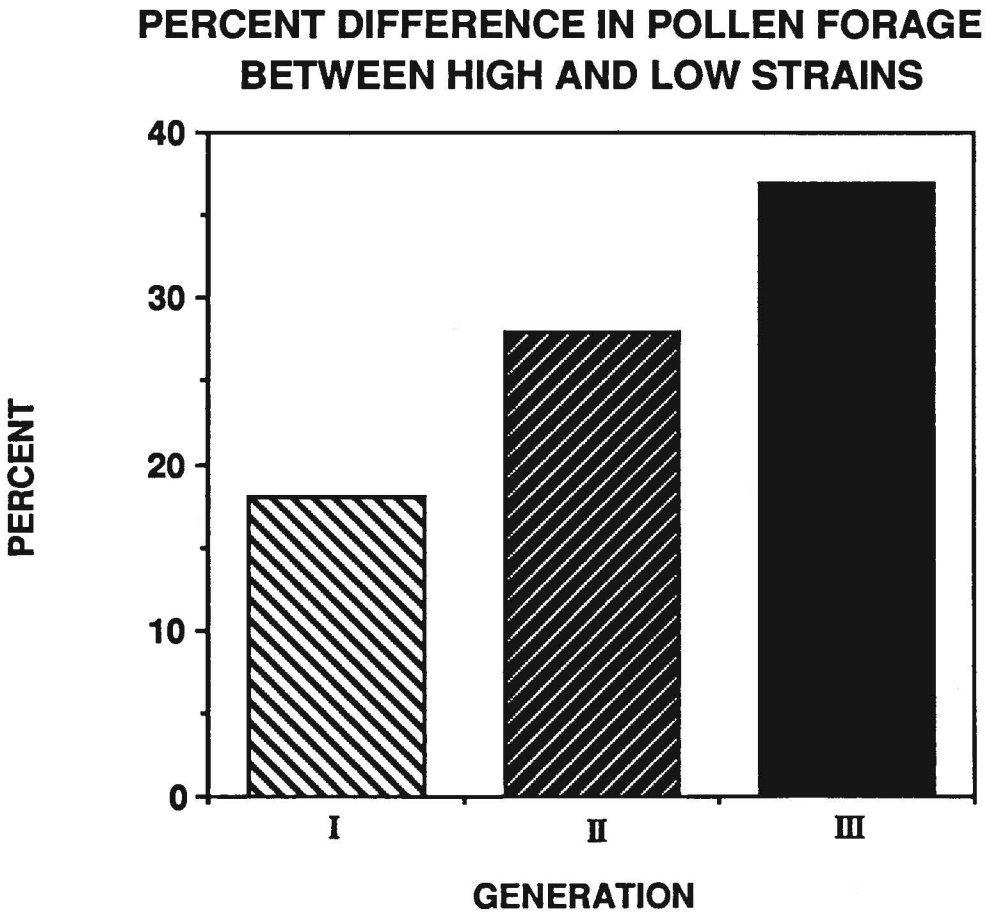

Figure 2.

Colonies from the high strain had nearly 40% more pollen carrying bees than those of the low strain (see Fig. 2) and a 102% greater ratio of pollen/nonpollen collectors. We now have produced our test colonies for

generation 4. These tests will take place in the almond orchards in the winter of 1992.

Objective 6: Commercial Test of Pollen Hoarding Stock

METHODS: 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 evaluated the pollination activities of the colonies in alfalfa fields.

RESULTS: In alfalfa, 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). The increased pollen collecting and brood rearing are important for beekeepers providing pollination services for

alfalfa. Lack of pollen collection and subsequent reduced brood rearing are major economic problems.

DATA RELIABILITY: We have not yet tested our high and low strains against "commercial" stock. Our strains were derived directly from available commercial stocks and we assume the lows are more like commercial stocks than are our highs. However, this will still have to be confirmed. We also don't know if having more pollen collecting bees results in more pollination. Again, this will have to be tested.

Objectives 1, 2, and 3: Defensive Behavior

METHODS: 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.

Another experiment was initiated to determine the efficacy of backcrossing AHB queens to European drones to decrease the defensive behavior of commercial and feral bees. Ten AHB queens were raised and each was instrumentally inseminated with the semen of a different European drone. From these queens, 11 daughters (F1 generation) were raised and also instrumentally inseminated with semen from European drones. Queens from this first backcross were then raised and likewise inseminated. All colonies of all generations were tested as described above and compared to "pure" European and AHB colonies.

RESULTS: The results of this study suggest that the extreme defensive behavior of Africanized bees is a dominant trait of individual workers (see Figs. 3 and 4). 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

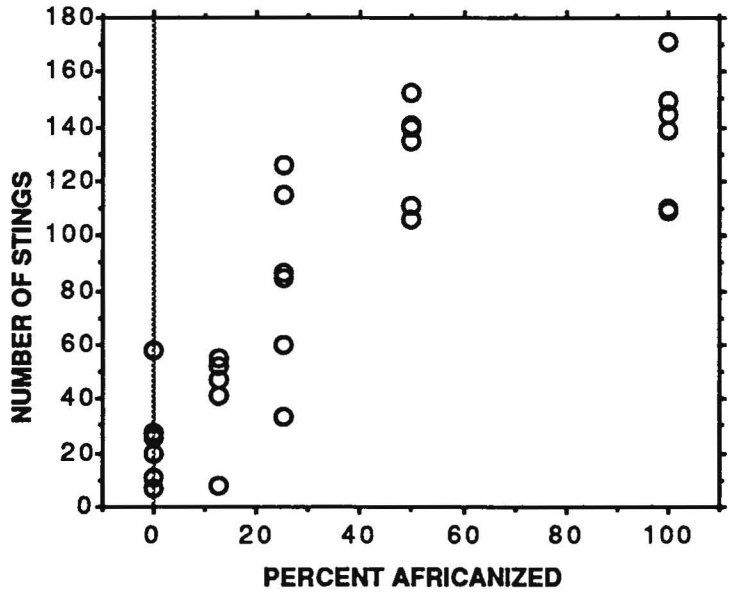

Figure 3.

Africanized and pure European workers gave defensive responses that were proportional to the proportion of hybrid workers in the colonies.

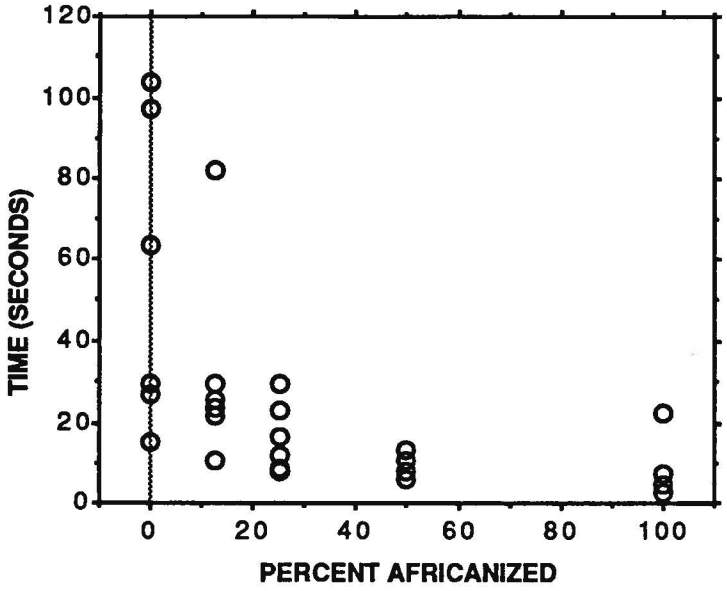

Figure 4.

Two generations of mating AHB to European drones restored a European-type response for number of stings. However, it took three generations to restore a European-type response for time to react to the patch stimulus (see Figs. 5 and 6).

DATA RELIABILITY: One caveat must be issued with these data. The genetic dominance relationships and the backcross results may be cross specific. Our European stock originally came from California, many years ago. There may be differences in stocks when combined with AHB.

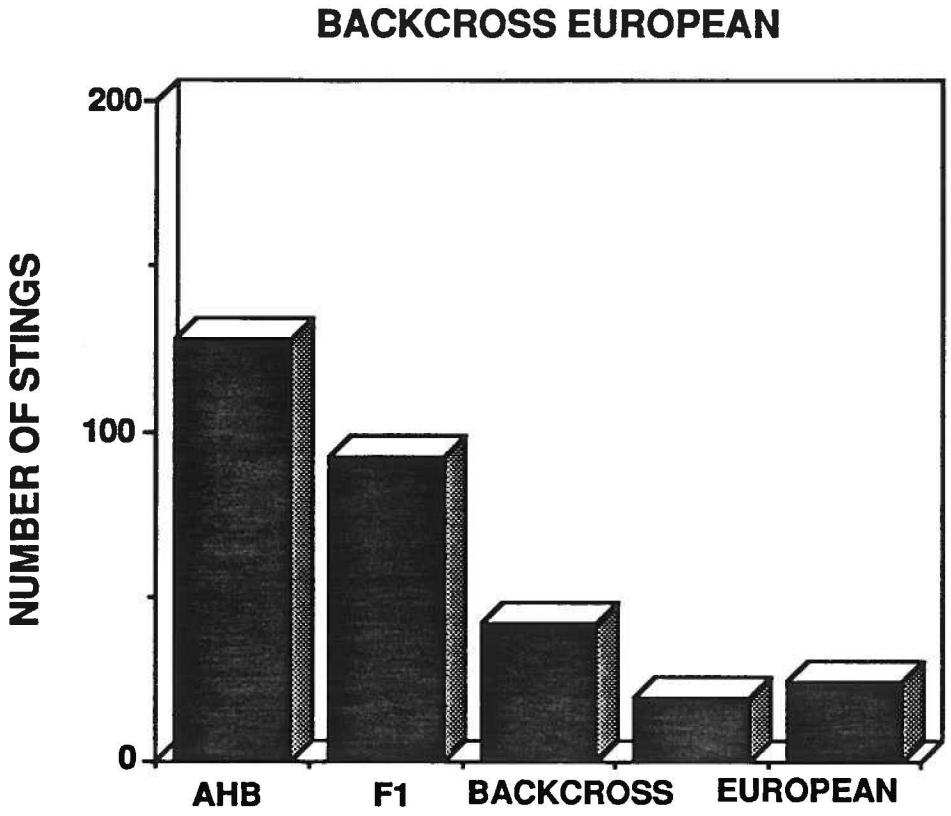

Figure 5.

Objectives 1, 2, 3, and 4: DNA Markers

METHODS: We continue to screen samples of bees from feral colonies collected in California, and Africanized bees from Mexico, for DNA polymorphisms that may be useful for identification. We have screened more than 600 random amplified polymorphic DNA (RAPD) markers.

RESULTS: So far we have not found any markers that are diagnostic for AHB. It isn't likely that any exist because of the hybrid origins of the bees. These results are supported by other labs using different DNA techniques. However, we have identified 10 markers that demonstrate considerable gene frequency differences between our sample groups and look very promising for making deteminations of the degree of Africanization of individual workers. We are now assaying individuals from 81 feral colonies from 30 different counties of California and 49 Africanized colonies from 34 locations

in Mexico and Central America to determine "allelic" frequencies of these different markers.

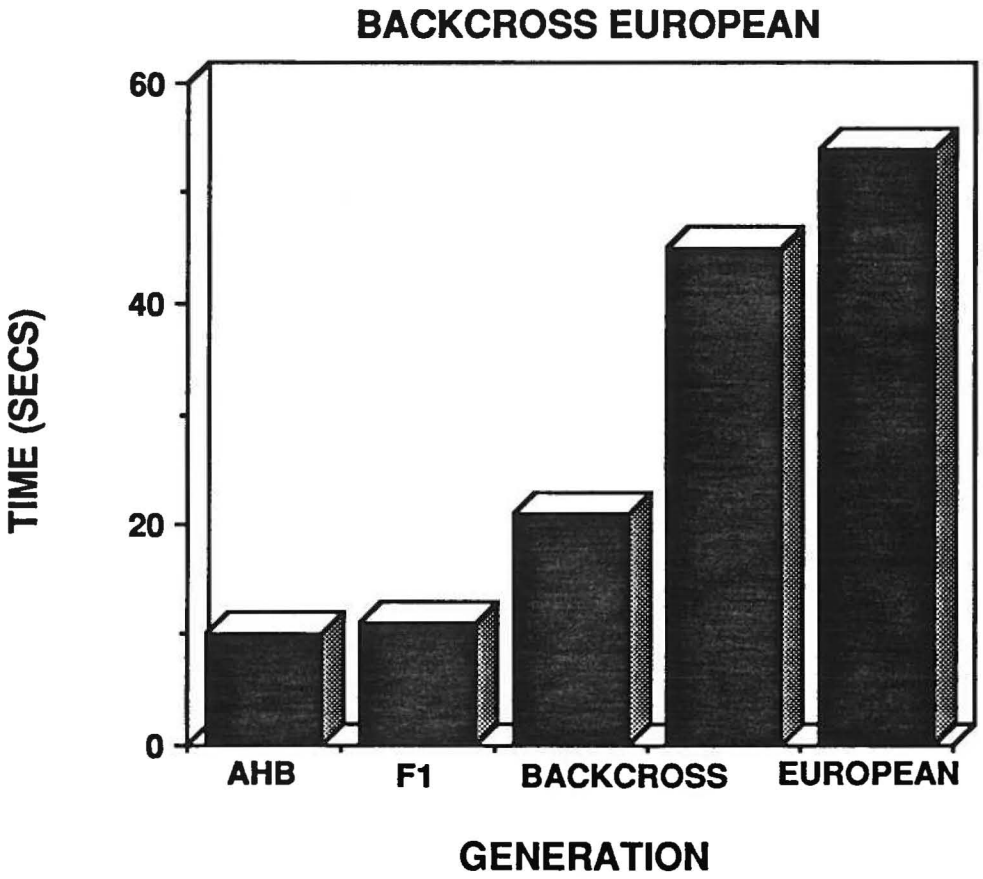

Figure 6.

DATA RELIABILITY: We have studied the patterns of inheritance and repeatability of these PCR reactions in great detail. These markers are resolved as bands on agarose gels, much like allozymes. Two parental bees, a haploid drone and diploid queen, were screened by using their genomic DNA as template for PCR with 68 different ten-nucleotide primers of random sequence. Differences for the presence of individual bands were scored between parents. An average of 6.3 bands and 1.3 polymorphisms for presence/absence were observed between the parents per primer. Thirteen of these primers were used to determine the inheritance of RAPD marker alleles

in their progeny and in drones from a daughter queen. Four types of polymorphism were observed. Polymorphisms for band presence/absence as well as for band brightness were inherited as dominant markers, appearing in Mendelian expectations in haploid and diploid progeny. Polymorphisms for fragment lengths were also observed. These segregated in a near 1:1 ratio in drone (haploid) progeny but could not be scored in diploids (queens and workers). Mixing of amplification products after PCR showed that these bands were the result of heteroduplex formation from DNA of heterozygotes. In two of the four cases of heteroduplex formation, the alternative alleles were manifested as small fragment length polymorphisms, resulting in codominant markers. The homozygote classes in the other two cases could be distinguished only by mixing the amplification products.

In summary, we now have confidence in the repeatability and readability of these markers. We know that they are Mendelian in inheritance, we know how to interpret the resulting banding patterns, and they should be valuable tools for assessing population composition and origins of exotic insect and plant pests.

V. IMPLEMENTATION IN THE FIELD

We have already implemented our breeding program in the field. We hope to eventually have a mechanism for producing and certifying commercially desirable, Africanized gene-free, honey bee stocks. This program will incorporate closed population breeding with instrumental insemination, identification of Africanized bee free regions of California by DNA analysis, mating queens in those "safe" areas, and distribution of stocks to queen producers and pollination service contractors.

Studies of defensive behavior are promising with respect to altering the defensive behavior of feral bee populations. What we now need is

research directed to determining the best way to maximize the mating success of European drones with AHB queens in specifically designated areas. The bad news from the defensive studies is that the high defensiveness appears dominant. That suggests that small amounts of introgression of AHB into European feral or commercial populations will result in great increases in stinging behavior.

DNA markers can be used as an additional diagnostic tool for determining Africanization of California honey bee populations. Currently, a single operator can run about 90 individual reactions per day. This is faster than morphometric determinations and nearly as fast as allozymes. We need to expand the allele frequency data base and continue processing our samples of California feral bees. We also need to sample California commercial bees. We have made site specific primers for determining mitochondrial DNA of European and African origins but have not yet worked out the technical details of using them. I think that these RAPD markers are the diagnostic tool of the future for all exotic pests.

VI. RECOMMENDATIONS

RAPD markers should be incorporated into all exotic pest diagnostic programs. They are fast, reliable (providing care is taken to learn their limitations), and relatively inexpensive. We still need to expand our data base for California bees in order to map the process of Africanization in California.

F1 hybrid bees have full AHB responses but are morphometrically European! I think this demonstrates a serious problem for any attempt to certify or regulate "bad" bees based on morphometric analyses. We are currently accumulating a data base in Mexico relating morphometrics to behavior.

VII. PUBLICATIONS

Page, R. E. 1992. How Africanized honey bees will affect California agriculture. *California Agriculture*: ~~in press~~. (46)1. pp 18-19.

Page, R. E., M. K. Fondrk, and G. E. Robinson. Selectable components of sex allocation in colonies of the honey bee (*Apis mellifera L.*). Submitted.

Hunt, G. J. and R. E. Page. Patterns of inheritance with RAPD molecular markers in the honey bee reveal novel polymorphism. Submitted.

There are many other potential publications based on data presented here but they are not yet in completed manuscript form.