

## **Project No. 97-RP-00 - Honey Bee Management, Genetics, and Breeding**

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### **Objectives:**

1. Develop management methods for the commercial beekeeping industry to maintain and produce commercial honey bees of good genetic stock that are resistant to diseases, free of objectionable Africanized honey bee genetic material, and are of high commercial value for pollination.
2. Selectively breed and maintain strains of bees that are more effective pollinating units.
3. Construct genetic maps and identify genetic markers that are close to genes of economic importance, such as defensive behavior, pollen collecting, and disease resistance. These maps will then be used in programs of dna marker assisted breeding and for directly assessing the potential characteristics of colonies of commercial breeding stock.
4. Conduct dna surveys of feral honey bee populations to determine the extent of the spread of Africanized honey bees in California.

### **Commercial Test of High Pollen Hoarding Bees**

This year we tested colonies derived from queens of: 1. our high pollen hoarding strains, 2. hybrids of a high x low strain cross, and 3. commercial bees that had not been selected for pollen hoarding or foraging. We have shown in the past that colonies with high strain queens that have naturally mated with males from commercial colonies collect and store more pollen than do colonies with unselected commercial queens. We have also shown that colonies that store more pollen have more pollen foragers. Our low pollen hoarding strain of bees store less pollen, have fewer pollen foragers, but are not significantly different from our high strain bees for the amount of honey they produce.

In cooperation with John Foster, a local beekeeper and queen producer, we produced and marked about 200 high strain and hybrid queens (100 of each). We also marked about 100 commercial queens as controls. The queens mated naturally in

commercial mating yards and were introduced into production hives in June, 1996. All colonies were managed under normal commercial conditions. The following February (1997) we relocated in almond orchards about 50 high and hybrid queens and an equivalent number of commercial queens in control hives. We evaluated these colonies and, as expected, colonies with naturally-mated high-strain queens stored about 3 times more pollen than equivalent control colonies. Colonies with hybrid queens stored the same amount of pollen as their commercial controls. All colonies had the same average amounts of stored honey and equivalent amounts of brood and adult bees. The only difference we observed was the quantity of stored pollen.

These results demonstrated that naturally-mated high-strain colonies have higher pollen foraging activity. However, more pollen foraging does not necessarily result in a decrease in honey production. Colonies with hybrid queens performed like unselected commercial colonies supporting our previous results that suggested the high pollen foraging trait is recessive in inheritance.

### **Controlling Pollen Foraging Behavior with Brood Pheromones**

Pheromones are chemicals produced by bees that affect the behavior of other bees. We have long known that the presence of brood in colonies stimulates foraging behavior in general and specifically increases pollen foraging. However, the mechanism by which brood results in these changes has been unknown. We rinsed honey bee larvae with a solvent that removed chemicals that adhere to their bodies. We then placed controlled quantities of these extracted chemicals (pheromones) into small colonies and observed their effects on foraging behavior. We found that numbers of active pollen foragers increased immediately in response to these extracts, in some cases more than 4 times. Nectar foraging behavior was unaffected. These results are very exciting because we now have two ways that we can dramatically increase pollen foraging in colonies. We have high pollen hoarding strains that have about 1.8 times more pollen foraging activity than commercial colonies, and we can add brood extracts and dramatically increase pollen foraging in commercial colonies, apparently without affecting their nectar collecting. Next we need to determine the specific chemical(s) responsible for this behavior and conduct field tests with commercial hives.

### **Mapping Genes for Pollen Foraging Behavior**

Recent advances in molecular biology have led to the ability to map genes responsible for observed variation in specific traits. We have constructed linkage maps of the honey using random amplified polymorphic DNA (RAPD) markers and have identified three regions of the honey bee genome that affect differences in pollen foraging behavior between our selected high and low pollen hoarding strains of bees. We previously reported two of these regions designated *pln1* and *pln2*. We have now mapped a third region, *pln3*, and have independently verified its effects. We have also identified some additional, interesting effects of two of these quantitative trait loci (QTLs). *pln2* and *pln3* appear to affect both the decision of bees to forage for pollen and

nectar and also the concentration of the nectar that they find suitable to collect. Individuals that inherit “high” alleles have a greater tendency to collect pollen than do individuals inheriting a “low” allele. This is also true for individuals with high and low alleles for *pln1*. However, individuals with high alleles for *pln2* and *pln3* that collect nectar will accept nectar with lower concentrations of sugar than will foragers with low alleles. As a result, the average concentration of sugar in the crops of returning foragers with low alleles is higher.

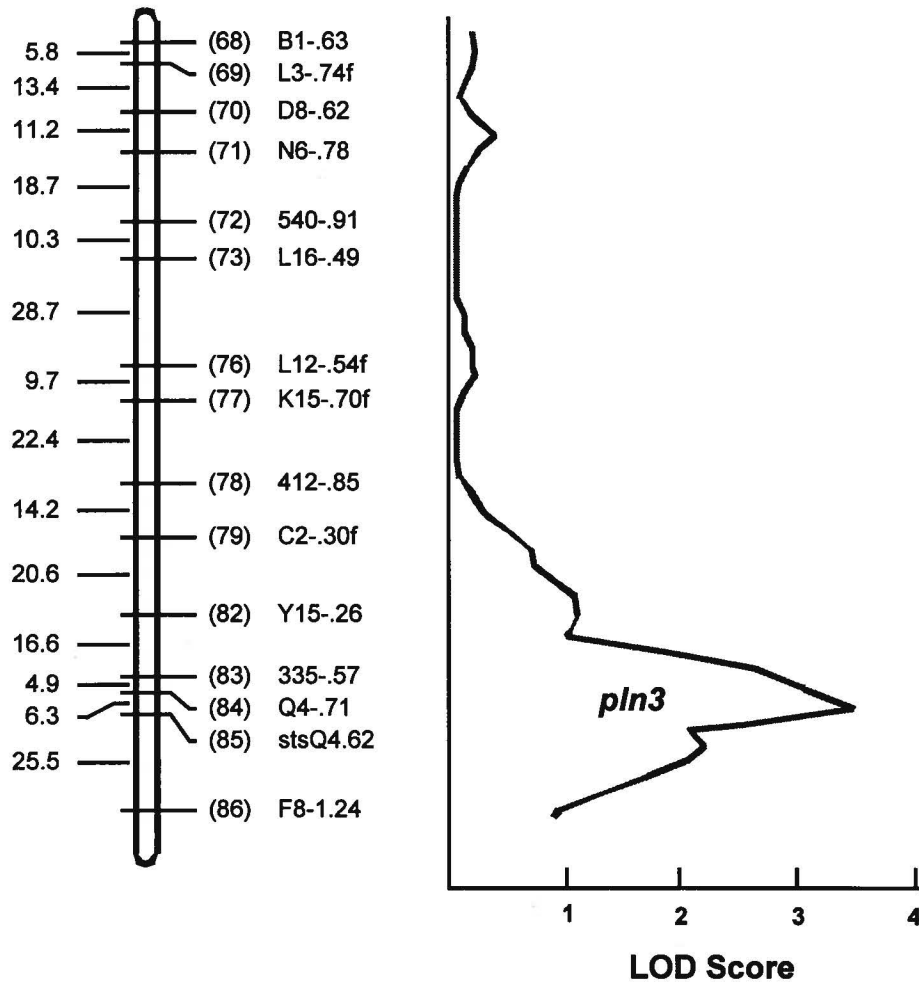


Figure 1. A linkage map of QTL *pln3*. The figure represents a chromosome. The numbers at the left represent the distance in centiMorgans (a measure of genetic recombination) between specific markers shown at the right. The line trace is a measure of statistical significance for the presence of a pollen foraging QTL in the different regions of the linkage map. A higher LOD score represents a greater likelihood that a QTL exists in that region. Any value above about LOD 3 is considered statistically significant, as is *pln3*.

We think this shows a fundamental relationship between the genes that we have mapped, the sensory physiology of the foragers, and their foraging behavior. Nectar and pollen foragers differ in their response thresholds to sucrose solution, which is an indicator of their abilities to discriminate among different concentrations of sucrose. We captured bees returning from foraging trips and restrained them in small brass tubes. We then touched their antennae with increasing concentrations of sugar solution. When the concentration exceeded the threshold of a forager she would extend her proboscis (tongue). Pollen foragers respond at lower concentrations than do nectar foragers. This difference also holds for high and low strain foragers that are raised together and collected from the same colony. High strain bees have lower response thresholds, even when they are foraging for nectar. These differences are detectable even in very young workers that have never foraged.

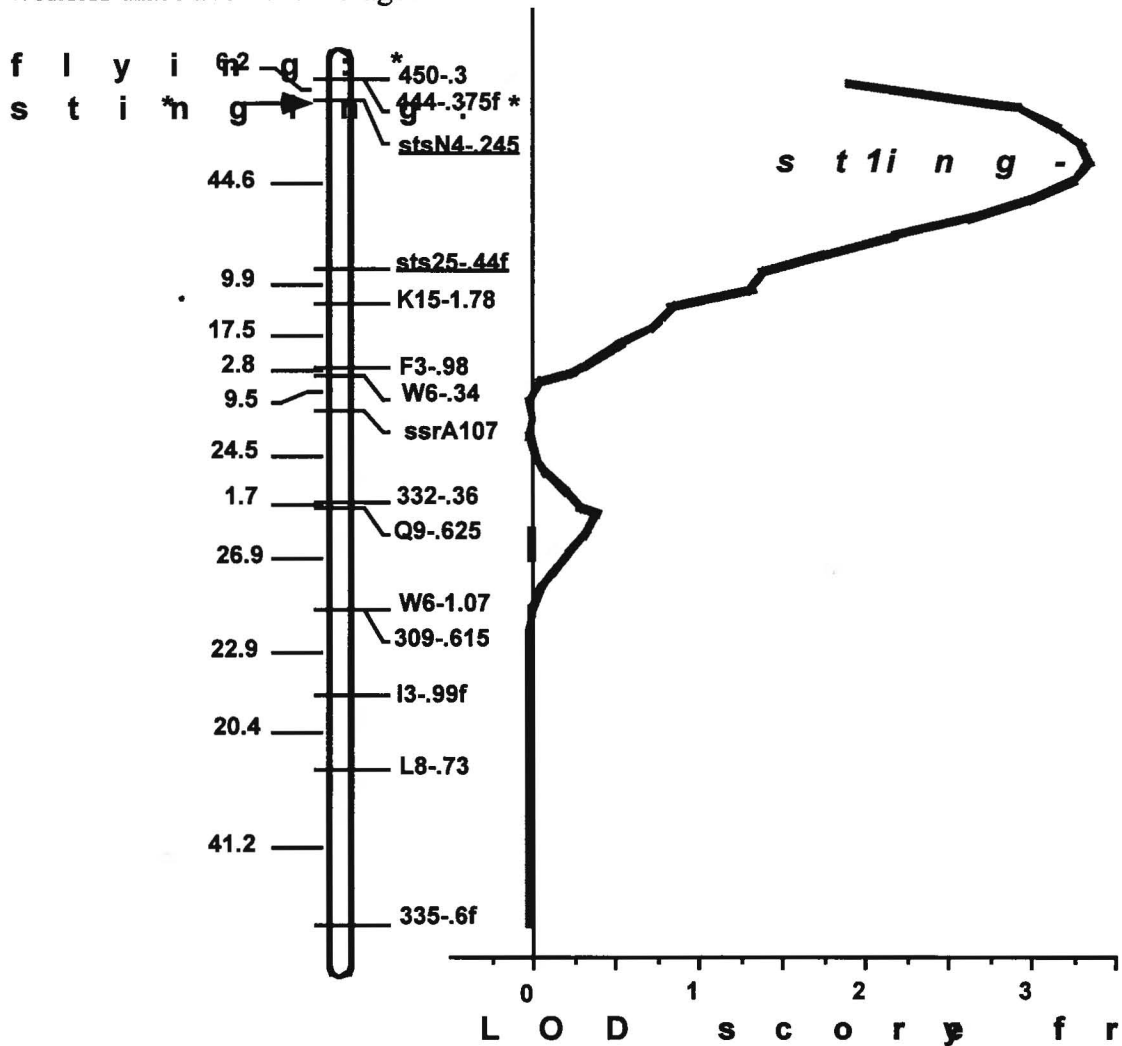


Figure 2. A linkage map of *sting-1*, a QTL affecting defensive behavior of Africanized honey bees.

### Mapping Genes for Defensive Behavior

In earlier studies we showed that the strong defensive behavior of Africanized honey bees in Mexico was a trait that demonstrated genetic dominance. We have subsequently done extensive gene mapping of the genome (genetic composition) of bees derived from Africanized and European sources. We have located one gene that is having a major effect on the observed difference in stinging behavior between European and Africanized bees. Next we will try to independently verify our results. If verified, we may be able to locate specific gene markers that will predict the defensive behavior of colonies.

### **The Spread of Africanized Honey Bees in California**

This Spring we collected honey bees on flowers in the south-eastern corner of California including Winterhaven, the Imperial Valley, along the west side of Salton Sea into the Coachella Valley, east to Blythe, west to Palm Springs, and south through Anza Borrego. We sampled 503 bees from 86 sites and examined the DNA contained in their mitochondria as a diagnostic for Africanized bees. Thirty-four samples had African-type mitochondria indicating that the bees were Africanized. This was the third year that we have conducted this random sampling and two trends are apparent. 1. The area containing Africanized honey bees is growing. This year we found Africanized honey bees outside of the area where they were found last year. New finds occurred in the Coachella Valley and Palm Springs. 2. The density of Africanized bees is increasing in these areas. In 1995, we found no Africanized honey bees using our method of sampling at flowers, suggesting that they were present in very low levels relative to European honey bees. In 1996, less than two percent of the bees we sampled were Africanized, while this year we had about seven percent.