FINAL REPORT

Project Title: The influence of time of year and rearing environment on the acceptance of introduced European queens in Africanized honey bee colonies.

Project Number: 02-GD-01 Correct Project Number: 03-GD-01

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Objectives

1) **Determine if the rate of success in introducing European queens into African colonies differs based upon time of year and amounts of pheromone emitted by queens.**
2) **Determine if rearing environment of queens influences their pheromone profile and acceptance into Africanized colonies.**

Objective 1. Colonies of European honey bees become Africanized if they replace their queen and she mates with African males (drones). The only way to reverse the Africanization process is to introduce a European queen that has mated exclusively with European drones. However, it is difficult to introduce European matriline queens mated with European drones into Africanized colonies and have them accepted.

In previous studies, we examined the chemical profiles of volatiles emitted from European and African queens. We documented that the profiles differ with respect to matriline, time of year and state of the colony. We designed an experiment to compare the success rate of queen introduction under three different colony conditions and chemical profiles of the queen. The first was during a honey flow when most of the comb in the colony is being filled with incoming nectar and space for the queen to lay eggs is limited. The second condition was when relatively little nectar was coming into the colony but the brood area was expanding (late June through July in Tucson). We supplied honey and pollen to the colonies so that brood rearing was not constrained by a lack of resources. The third condition was in the fall when brood rearing is on the decline and the colony is preparing for winter. We conducted the experiments using 5-frame nucleus colonies. We sampled the volatiles emitted by the queens just prior to their introduction and 6 weeks after they were introduced into the colonies. We focused on a particular compound (hereafter referred to as Compound-A) that our previous studies have indicated is associated with egg lying. The experiment was run for 6 weeks because by that time the colony population was composed almost entirely of offspring from the introduced queen.

Results

On average, amounts of Compound-A in queens increased 440% after laying eggs (Fig. 1 QL) in colonies compared to the initial levels we measured when the queens arrived in mailing cages where they had been confined for 3-5 days (Fig. 1 QC).

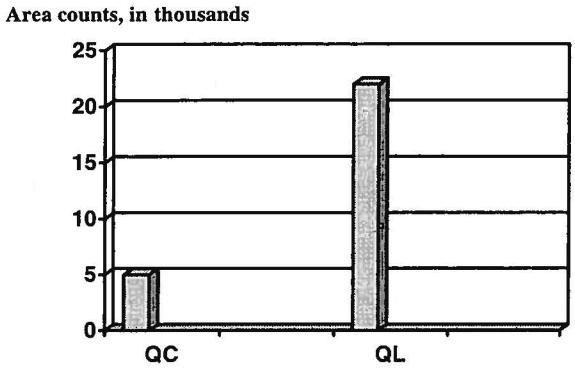

Figure 1. Amounts of Compound-A in queens that had been confined in shipping cages for 3-5 days (QC) and those that had been laying for 6 weeks (QL).

The amount of Compound-A emitted by queens in the Honey Flow trial was about twice as high as during the July or Fall trials (Table 1). There appears to be an inverse relationship between the amount of Compound-A emitted by queens and the amount of brood in the colony. During a honey flow, there were fewer frames with developing bees and more frames with nectar and honey than during the July or Fall trials when 60% or more of the comb in the colony contained brood.

Table 1. Comparisons of amounts of Compound-A between Trials-1 and 2 (queens laying for 6 weeks)

Trial	Colony Type	Queens Sampled	Average Amount of Compound-A (k counts)
Honey flow	EHB	6	20.7 ± 6.4
	AHB	5	22.4 ± 10.4
July	EHB	8	9.6 ± 3.1
	AHB	4	10.5 ± 3.4
Fall	EHB	8	7.6 ± 1.2
	AHB	7	8.1 ± 3.1

The acceptance rate of the queens in Africanized colonies was not significantly different at the 0.05 level between Africanized and European colonies during the Honey Flow ($t = 0.29$, d.f. = 7) or Fall ($t = 1.11$, d.f. = 7) (Table 2). However, during the July Trial, the acceptance rate of queens in Africanized colonies was significantly lower than in the European colonies ($t = 2.82$, d.f.= 8). The data suggest that the acceptance of European queens in Africanized colonies is greater during a honey flow or in the fall. There does not seem to be a relationship between amounts of Compound-A emitted by queens and their acceptance rates in colonies.

Table 2. The percentage of successful introductions of European queens into colonies under different conditions. Queens were introduced into 8 colonies of each colony type

Trial	Colony Type	% of queens accepted
Honey flow	EHB	75
	AHB	62
July	EHB	100
	AHB	50
Fall	EHB	100
	AHB	87.5

Objective 2. Queens were removed from European and African honey bee colonies to stimulate the hives to rear new queens. We also added a frame from the African colony into the European colony, and a frame from the European colony into the African colony to determine if workers would rear queens from unrelated brood. Queen cells reared from brood in cells on the frame will hereafter be referred to as 'natural cells'. We also grafted worker larvae less than 48hrs. old from the African and European colonies, placed them in queen rearing colonies for 24 hrs, and then removed them and put the cells in the queenless African and European colonies. We placed the grafted cells in the colonies to increase the number of queens reared in both colony types. The procedure described above was repeated three times.

When the queens were <24hrs from emerging, we removed the cells from the colony and placed them in incubators set at hive temperatures. 48hrs after the queens emerged we sampled volatiles emanating from them using a solid phase microextraction device. We analyzed the sample using gas chromatography and mass spectroscopy

Results

Queenless European colonies reared 72.7% of the grafted European queen cells we placed in the colony (n = 22) and 93% of the African cells (n = 28). The African

colonies reared 94% of the grafted African cells (n = 18) and 100% of the European cells (n = 18) we placed in the colonies. The European colonies reared eight natural queen cells on frames with European brood and four cells on the frames from the African colony. The African colony reared four natural cells on African frames and eight cells on the European brood frames we placed in their colonies.

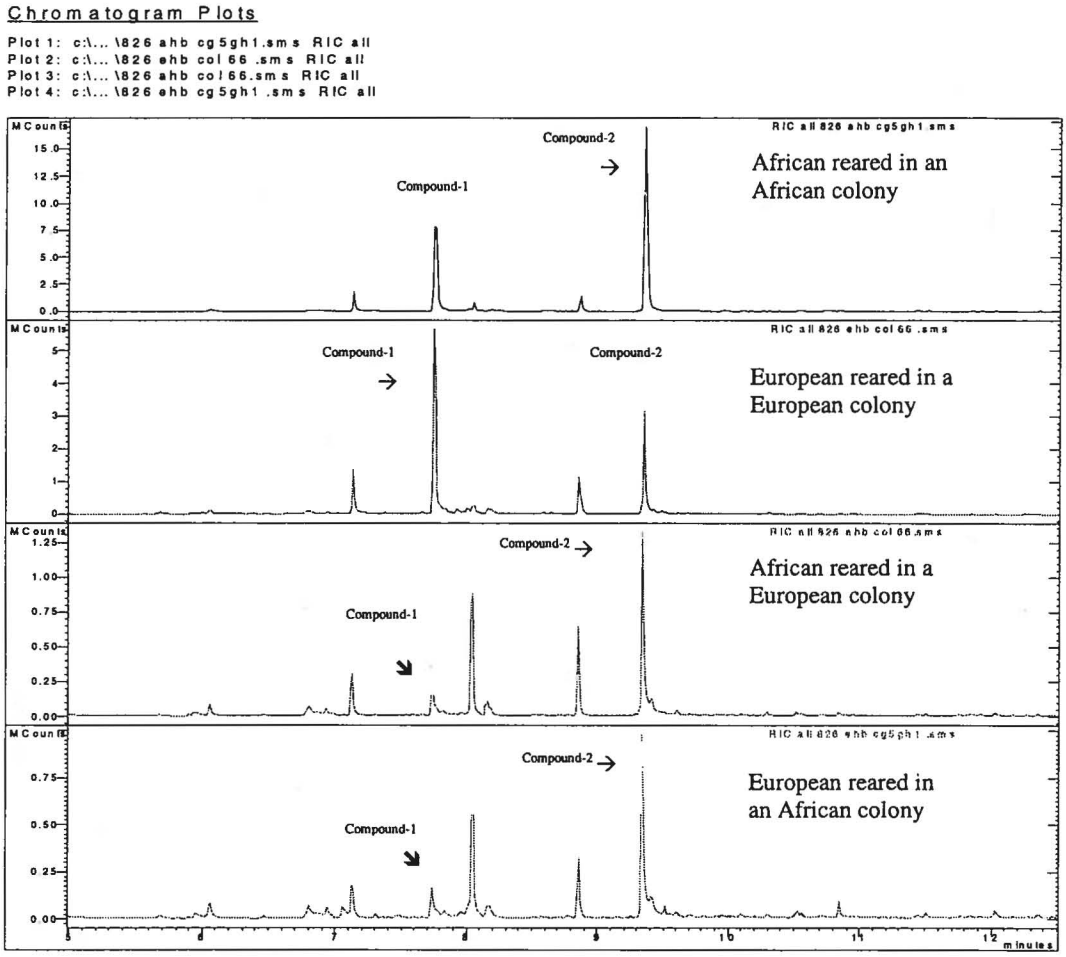

Figure 2. Chromatograms of volatile collected from virgin honey bee queens reared in different colony environments.

Rearing environment and queen type influenced the volatile compounds emanating from queens. African queens reared in African colonies had higher amounts of volatiles compared with European queens reared in European colonies. African queens had lower ratios of Compound-1 compared with Compound-2 while in Europeans the ratios were reversed. Queens reared in colonies other than their own had very low levels of volatiles (especially Compound-1). The profiles from African and European queens were more similar to each other than to either queen type reared in their own colony.

Conclusions

Results from this study indicate that colonies that have become Africanized can be requeened with a mated European queen but the time of year will affect the success rate. We had the greatest success when we requeened in the fall.

Our study indicates that beekeepers have options for reversing the Africanization process other than introducing a mated European queen if a colony becomes Africanized but can be placed in a non-Africanized area. Africanized colonies will readily rear queens on frames of European brood that are placed in their colonies. If a colony has an African queen, the beekeeper can remove her and add frames of European brood that the workers can use to rear a new queen. If there are no African drones in the area, the new European queen will open mate with only European drones and the Africanization process will be reversed.

During the course of this study, we isolated and identified a queen-specific volatile compound (Compound-A) that appears to respond dynamically to conditions that influence the egg laying rate of a queen. When a queen is confined and cannot lay eggs, levels of Compound-A are very low. The levels rise when she begins to lay eggs, but the degree of Compound-A increase appears to depend upon the amount of brood in the colony. Levels of Compound-A are relatively lower when the colony has many frames of brood compared with times when there are fewer brood cells. Currently, we are conducting experiments to determine the relationship between queen egg laying rates and amounts of Compound-A produced by queens. We also are conducting behavioral bioassays to determine worker responses to low and high levels of Compound-A.