Year-End Report
Project Year 2004

**Project Title:** The role of volatile chemicals in the usurpation of European honey bee colonies by Africanized honey bee swarms

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## The Problem:

Honey bees are essential for the production of almonds. All commercial cultivars are self-incompatible and require cross pollination for nut set. In the past, the major challenge for beekeepers has been getting strong colonies to the orchards for the start of almond bloom. However, the immigration of Africanized honey bees (AHB) into California has created a completely new set of challenges for beekeepers and almond growers. AHB can mount fast defensive responses with large numbers of bees; up to 10% of the colony population will leave the hive to defend it within the first 30 seconds of perceiving a threat (DeGrandi-Hoffman et al 1998). If the threat persists, all the adult workers will eventually participate in defending the colony. This defensive response of AHB colonies can be life-threatening to humans and their pets and livestock, making the use of these bees untenable in many locales. Thus, to sustain current use of honey bees in agriculture we need to develop methods of preventing Africanization of European honey bee (EHB) populations. This has proven a challenge because Africanization occurs by multiple mechanisms and because even the transport of EHB from, through, or into areas with Africanized bee populations can quickly spread African characteristics.

One means by which Africanization occurs is the usurpation of European HB colonies by swarms of AHB. These small swarms cluster near the entrance of a European hive, penetrate the colony's defenses, and eliminate the European queen, replacing her with their own queen (Danka et al., 1992, Vergara et al., 1993, Schneider et al., 2005). Because all future reproduction will be by the Africanized queen, usurpation results in complete Africanization of the European host colony. This feature of Africanization by usurpation, along with the fact that EHB do not usurp AHB colonies, may partly explain the observation that in areas colonized by AHB, EHB genes and traits largely disappear rather than reaching a stable hybridization with AHB genes and traits (Hall, 1999; Segura, 2000; Clarke et al., 2001; 2002). Usurpation rates of managed EHB colonies vary by region and season, but we recorded annual rates in southern Arizona of 12 – 30%. This high rate coupled with the complete Africanization mentioned above, makes usurpation a significant problem. This is particularly true for bees used to pollinate almond trees. These bees are moved onto "ranches" in Southern California several months prior to being used for pollination to avoid transport in winter weather conditions. The bee ranches are located in areas with feral AHB populations, making these temporary apiaries prime targets for usurpation. Further compounding this problem is that the colonies are present on the ranches during a period of intense seasonal usurpation activity; more than half of all usurpations occur during the months of October, November, and December (Schneider et al, 2005).

**The Solution:**

Preventing usurpation of EHB colonies is complicated by the difficulty of detecting usurpation. The swarms can be hidden underneath a hive's entrance board or mistaken for clumps of host-colony workers. In addition, the usurpation process can be completed within a few hours or overnight (personal observation), making it easy to overlook. Effective prevention measures will require knowledge of nest usurpation beyond the basic documentation of the phenomenon that currently exists (Danka et al., 1992; Vergara et al., 1993; Schneider et al., 2004). We know very little about how these relatively small swarms are able to successfully locate and usurp entire colonies. We know even less about how AHB swarms sometimes fail in their attempts to usurp EHB colonies, which is perhaps equally important for prevention of usurpation. Our goal is to learn both how AHB swarms often succeed and how they sometimes fail in usurping EHB colonies.

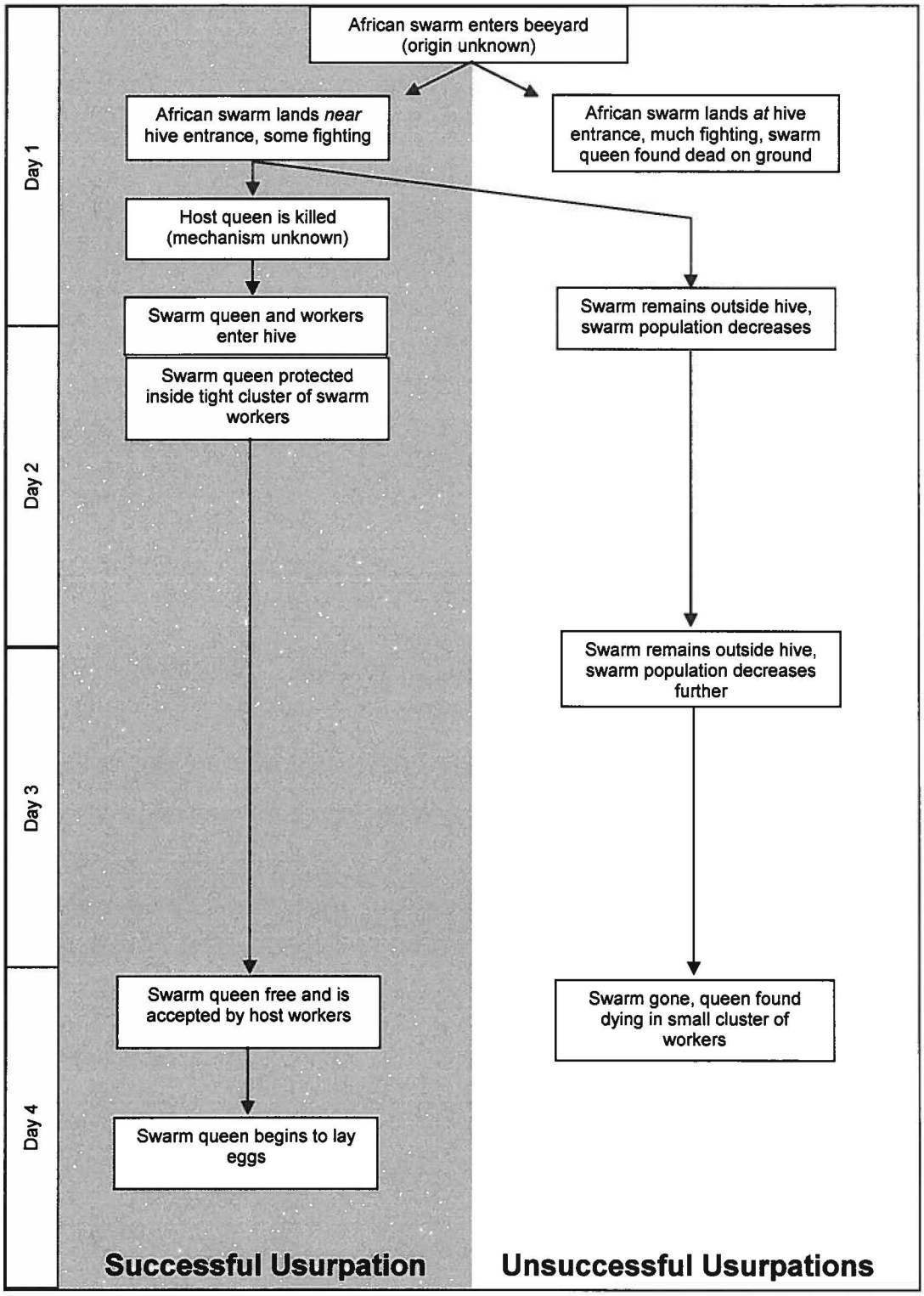
Chemicals produced by the bees are likely to play a major role in the success of nest usurpation. Though we know little about nest usurpation in honey bees, the phenomenon also occurs in other social insects such as ants and wasps. Nest usurpation in these taxa is often accomplished by mimicking the chemical identity of the host-colony members (thus preventing attack by nest guards), and then executing the host colony's queen (Hölldobler and Wilson, 1990; D'Ettorre et al., 2002). In honey bees, the production of chemical signals or the detection of chemical cues is likely to have three uses during usurpation: 1) locating a susceptible host colony, 2) evading host defense mechanisms, and 3) replacing the host colony's queen. Understanding these communication systems should allow us to tap into and disrupt the usurpation process in a safe and sustainable manner. Below, I describe our accomplishments studying nest usurpation during the 2004 funding cycle.

**Summary of accomplishments from 2004 funding cycle**

Between August 1, 2004 and July 1, 2005, we had approximately 32 Africanized swarms enter our apiary, 7 of which attempted to usurp the European colonies that were located there. This number was significantly below our projected number of usurpations (23), which was based upon records of our apiary from previous years (published by Schneider, Deeby, Gilley, and DeGrandi-Hoffman, 2004). The reason for this shortfall is unclear, but it appears that environmental conditions did not favor usurpation. As a result, the number of samples was lower than is needed to fully address some of our objectives. However, we were able to learn much about usurpation swarms despite this shortfall. Below I list the stated objectives from the 2004 proposal (*in italics*), together with a brief report of our conclusions with regard to each objective.

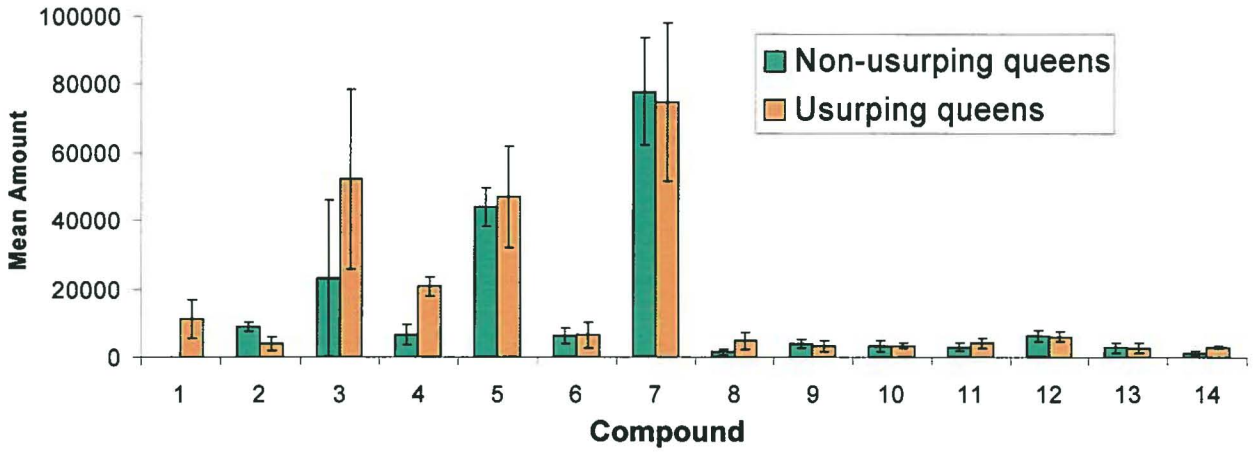
*1. Describe the events within a hive during successful and unsuccessful usurpations*

We were able to observe several usurpations closely in observation hives, allowing us to determine the basic sequence of events during usurpation. Below is a flowchart that illustrates the sequence and approximate timing of these events.



*2. Determine whether the volatile profile of a usurping queen affects her chances of acceptance by the host workers*

We measured the volatile profiles of usurping and non-usurping queens and found no significant differences in mean amounts of 14 chemical compounds (see figure below). Usurping queens do not therefore have a unique volatile profile that facilitates acceptance by the host workers.




*3. Determine whether the volatile profile of a host queen affects her chances of survival*

We did not have enough usurpation attempts on queen-right colonies this season to compare successful vs. unsuccessful with respect to host-queen volatiles. We should be able to address this objective with one more year of data. Meanwhile, however, we have increased our chance of success by compiling a data base of the volatile profiles of over 70 European queens. This database has shown us the characteristics of typical volatile profiles and how profiles vary between queens, allowing us to more easily detect unusual profiles that might be related to usurpation.

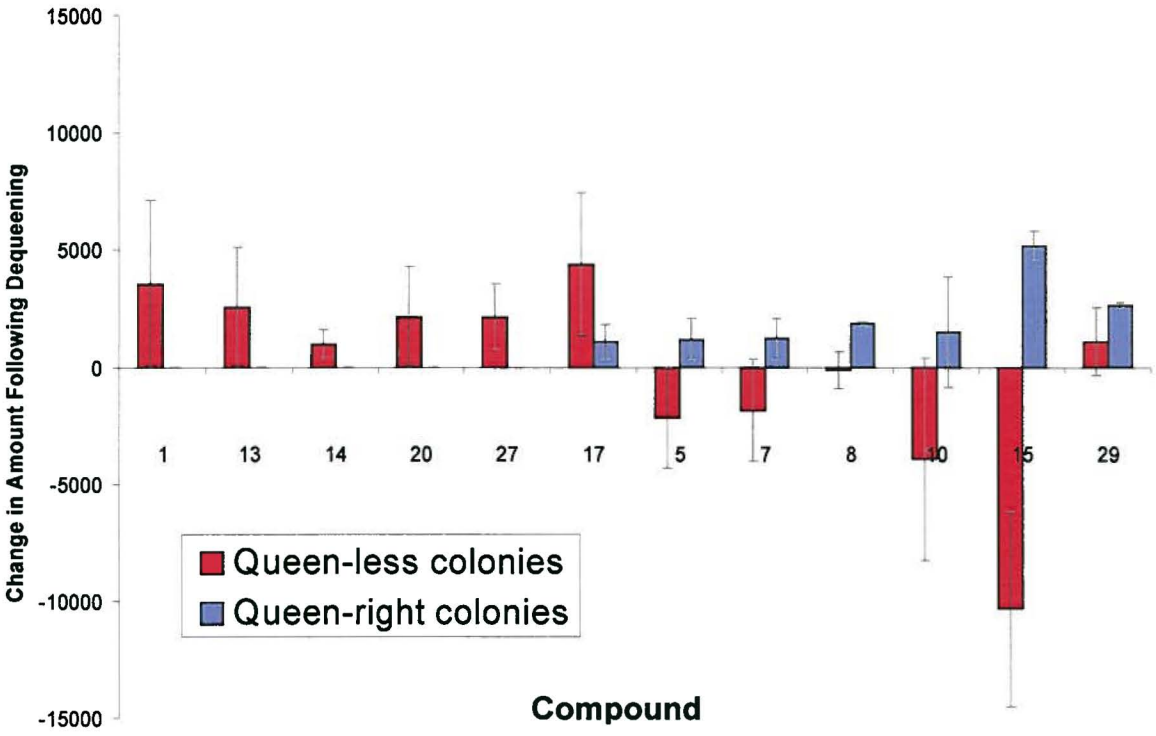
*4. Determine if high usurpation rates of queen-less colonies are due to a greater number of usurpation attempts, greater success of each usurpation attempt, or both*

Evidence to date suggests that queen-less colonies are targeted as much as queen-right colonies, but that usurpation attempts are more often successful on queen-less colonies. Additional data will show whether this trend continues.

*5. Determine whether a colony's volatile profile affects the probability of usurpation attempts or the probability of usurpation success*

This objective, using the methods described in the 2004 proposal, proved to be unrealistic in practice given the number of usurpation swarms entering the apiary. Volatile levels were unexpectedly low within the hives, forcing us to increase our sampling time. With the new sampling time (30 min), sampling colonies for 40 hours a week for 7 months would have yielded only about 5 data points! The project plan for 2005 takes this experience into account and includes a new method for addressing this objective that is likely to succeed.

However, we were able to establish by measuring the volatile profiles of queen-less and queen-right colonies, that differences in their volatile profiles exist which could explain why queen-less colonies are targeted more often and/or why usurpations are more successful against queen-less colonies. The figure below shows 6 compounds that *increased* in amount following dequeening and 6 compounds that *decreased* in amount following dequeening. Usurpation swarms could use these differences in volatile profiles as cues to locate susceptible colonies.



**Conclusions:**

The success of a usurpation attempt seems to depend on several factors. First, the exact location upon which the swarm lands (relative to the hive entrance) appears to be important for avoiding a mass defensive response from the host colony which leads to death of the usurping queen. Second, the number of queens in a usurpation swarm can affect the chance of success; swarms with multiple queens, though relatively rare, seem to have a better chance of success. Third, poor weather (e.g., cold temperatures and rain – especially in the fall) can decrease the chance of success, as it prevents swarms from moving into the host hive, eventually causing the swarm to run out of food. We currently do not have direct evidence that volatile chemicals affect usurpation success, but we have at least shown that differences in volatiles exist between queen-right and queen-less colonies and that the latter are easier to usurp. This is compelling because nature typically takes full advantage of such differences when they exist in biological systems like this.

Overall, our understanding of this complicated phenomenon is still crude, but I think I can honestly say that we've made more progress in the past year than anyone ever before. However, our knowledge is not yet adequate to design products or practices that will prevent Africanization via nest usurpation beyond general recommendations to beekeepers as to the common signs that usurpation is occurring or has occurred. These recommendations may allow beekeepers to requeen or destroy the effected colonies.

**Other accomplishments:**

In addition to addressing our stated objectives, the results obtained using the funds provided by the Almond Board of California during the 2004 project year increased our chances of gaining additional funds for the study of nest usurpation. In particular, the results presented above served as core preliminary data for a large (4-year) grant proposal on the topic submitted in June, 2005, to the National Science Foundation in cooperation with Dr. Stan Schneider of the University of North Carolina. The conclusions generated from this study, if granted, will also benefit beekeepers and almond producers.