A practical means for distinguishing africanized honey bees *(Apis mellifera L.)* from european honey bees by using central excitatory state, appendage mobility and sting viability

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This research is supported by a grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

Recebido para publicação: 24/11/2005
Aprovado para publicação: 24/05/2007

ABSTRACT

A key to understanding the expansion of Africanized honey bees (*Apis mellifera*) is distinguishing this aggressive form from its European relative. Current identification techniques have a degree of success, but each has its own set of problems, prohibiting wide-spread adoption and use. This study examined aspects of central excitatory state, persistence of mobile appendages, and a viable sting after decapitation for their use in distinguishing between these two races. Central excitatory state was not useful in distinguishing the Africanized honey bee from the European honey bee; however, appendage mobility and sting viability were significantly different between the two subspecies. Appendage mobility and sting viability are useful techniques for distinguishing the two subspecies, and alleviate the issues of expense, application, and accuracy.

Key words:
Classical conditioning. Behavior. Worker honey bee. Africanized honey bee. European honey bee.

Introduction

The purpose of this paper is to provide data on whether central excitatory state, mobility, and sting viability can be used to identify Africanized from European honey bees, *Apis mellifera* (Apidae: Hymenoptera). Aspects of central excitatory state, persistence of mobile appendages, and a viable sting after decapitation were tested for their utility in distinguishing between Africanized honey bees and European honey bees. The rationale for using these three behavioral characters is that they are easy to use, not apparatus intensive, and are easily quantifiable. We wanted to devise a method of identification that could be used by beekeepers and the general public. Our methodology would provide the general public and beekeepers a system for continuous monitoring of the status of their hives and would ultimately increase the level of public safety and awareness without relying on extensive training and expensive equipment.

Central excitatory state refers to the temporary "excitement" generated in invertebrate nervous systems by an unconditioned stimulus, such as sucrose [1]. Honey bees, for example, will show a temporary acceptance of an initially unpalatable solution after experience with sucrose. Mobility is defined as movement of any body appendage or tagmata. Sting viability is defined as the capability to project the stinging apparatus. These techniques are simple and do not require specialized equipment or training, so they should be useful to the general public.

Current techniques of identification include discriminant analysis of morphometric characteristics [2,3], gas chromatography [4], electrophoresis [5,6], FABIS [7], FABIS II [8], and the use of mitochondrial DNA [9].

These techniques have a degree of success, but each has its own set of problems, prohibiting wide-spread adoption and use [10,11]. For example, each technique contains analytical problems that increase the difficulty of analysis and they require trained technicians along with the use of well-equipped laboratories. In sparsely populated areas, resources do not exist to produce highly accurate Africanized honey bee sightings [12].

Bees were collected in glass vials 24 h prior to data collection and the vials were placed on ice to induce an unconscious state. Once unconscious, bees were secured into metal harnesses (10 mm by 28 mm). Harnesses were made from converted .38 special caliber shell casings with a 10 mm by 10 mm section removed from the sides [11,13,14]. Bees were held in harnesses by placing their heads on the upper rim and securing a piece of duct tape 2 mm by 30 mm behind their head. The bees were given time to regain consciousness and then were fed a 1.8 m sucrose solution. Excess sucrose was wiped from the head and antenna with a wet paper towel, and the bees remained in the laboratory for 24 h prior to experimentation to allow for habituation to the harness and the laboratory environment.

Material and Method

Subjects

Two colonies of honey bees (one for each subspecies) were maintained throughout the duration of this study. The Africanized colony (*A. m. scutellata*) was maintained in the apiary of the Laboratório Apícola of the Universidade Federal da Paraiba (UFPB), Bananeiras, Brazil. The Laboratório Apícola maintains several colonies suitable for research. The Bananeiras UFPB campus is an agricultural university located in the northeast portion of Brazil. The university maintains citrus groves approximately 400 m from the hives. In addition to citrus groves, the vicinity of the laboratory is heavily populated with various flowering species and is located near the Atlantic forest. Tests were run on these bees in July and August 2002. The Laboratory of Comparative Psychology and Behavioral Biology at Oklahoma State University in Stillwater, Oklahoma maintained the European colony (*A. m. mellifera*) . This colony had access to various woody and herbaceous species maintained on the University's campus. Both colonies have been used in previous research [11,13,14]. Tests were run on these bees in August and September 2002.

Conducting and comparing research from data collected on two different continents presented issues in analysis, but was necessary to conclude if the aforementioned characteristics were to be tested for distinguishing these two subspecies. To progress forward in the identification process, initial work had to be performed at the points of the least introgression (U.S. and Brazil). Separate continents introduced seasonal, climatic, available forage, and anthropomorphic variables into this study. These variables were controlled for as much as possible by collecting all data in months under similar climatic conditions.

Method
Central Excitatory State

Sucrose-induced central excitatory state has been observed to cause an increase in responsiveness to neutral stimuli for other invertebrate species, such as the blow fly *Phormia regina* (Diptera: Calliphoridae) and fruit fly *Drosophila melanogaster* (Diptera: Tephritidae) [15]. The response observed in this study was the Proboscis Extension Reflex (PER). Central excitatory state was induced by sucrose presented on 10 mm by 3 mm strips of Whatman® category 1004 150-mm diameter filter papers. Prior to presentation, each filter paper strip was saturated with a 1.8 m sucrose solution or water, depending on the trial. Sucrose and water solutions were presented by touching the saturated filter strips to the bee's antenna. Each bee received one exposure to the stimuli at an

assigned time interval. Solutions were presented to contralateral antenna to avoid mixing of the solutions. The first presentation to the left or right antenna was randomly assigned between bees. Once presentation took place, a positive response was evident by proboscis extension. A positive response was recorded as a '1' and a negative response as '0'.

Mobility and Sting Viability

The observation of mobility took place after decapitation occurred with the use of forceps to remove the head. After decapitation, bees were placed in one ounce POLAR® plastic cups for the allotted time then moved with forceps to a rubberized surface. If the bee was able to move its body or appendages, it was considered mobile. While on the rubberized surface, forceps were used to stabilize the bee and allow for the stinging behavior to take place (Figure 1). Care was taken so as not to force the stinging apparatus out of the body and cause a potential bias in data collection.

A viable sting (i.e., positive response) was scored when the stinging apparatus remained in the rubberized surface or by projection of the stinger out of the bee's abdomen. Responses were recorded in the same fashion as experiment one with the use of '1' for a positive response and '0' for a negative response.

Analysis

Data were statistically analyzed using SAS®, version 8.0. Contingency tables and probit regression models were used to detect differences between treatments. Contingency tables were used for each time period to see if a significant difference existed between subspecies for the ability to respond at that particular time interval. The use of contingency tables accounted only for variation at a specific time interval between both subspecies. For these techniques to be useful to the public, trends over time must be accounted for. Probit regression models were used if the contingency tables concluded significant differences between subspecies at specific time intervals. These models were used to detect subspecies differences and trends in the dependent variable over time.

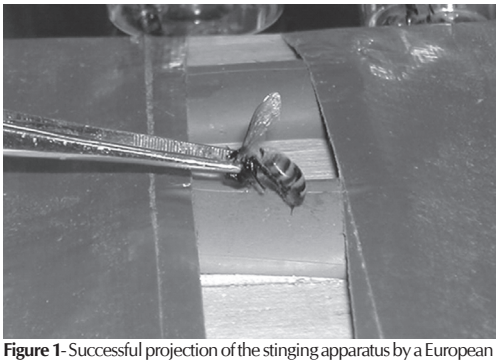

Figure 1- Successful projection of the stinging apparatus by a European honey bee (*Apis mellifera*) 8 hours after decapitation

Experiment 1: Central Excitatory State

Seven hundred bees were collected. Three hundred fifty bees were European and the remainder Africanized. Of the 350 bees for each subspecies, 175 bees were control (water only) and the remainder test subjects (sucrose and water). The 175 bees in each group were subdivided into seven groups of 25 bees each, which represent specific time intervals (1.5 sec, 1 min, 2 min, 4 min, 6 min, 8 min, and 10 min) of observation. Water only was used as the control to record a base rate of response for each subspecies of bee because it does not cause excitation in bees [16]. The base rate of response and the test results were then compared for evidence of a significantly greater frequency of the proboscis extension reflex for Africanized honey bees after sucrose induced excitation. Bees were tested daily to account for calendar variables such as temperature, atmospheric pressure, and age.

Bees were collected in glass vials 24 h prior to data collection and the vials were placed on ice to induce an unconscious state. Once unconscious, bees were secured into metal harnesses made from converted .38 special caliber shell casings with a 10 mm by 10 mm section removed from the sides [11,13,14].

Bees were held in harnesses by placing their heads on the upper rim and securing a piece of tape 2 mm by 30 mm behind their head. The bees were given time to regain consciousness and then were fed a 1.8 mol sucrose solution to satiation. Twenty-four later they were tested. The rationale behind the 24 h period was to allow for habituation to the harness and the laboratory environment.

After 24 h, the bees in each treatment were randomly divided into seven groups based on the amount of time that elapsed between presentation of a sucrose solution and presentation of water, or between presentations of water for the control group: 1.5 sec, 1 min, 2 min, 4 min, 6 min, 8 min, and 10 min. Sucrose and water were presented on 10 mm by 3 mm strips of Whatman® category 1004 150-mm diameter filter papers. Prior to presentation, each filter paper strip was saturated with a 1.8 mol sucrose solution or water, depending on the trial. Sucrose and water solutions were presented by touching the saturated filter strips to the bee's antenna. Each bee received one exposure to the stimuli at an assigned time interval. Solutions were presented to contralateral antenna to avoid mixing of the solutions. The first presentation to the left or right antenna was randomly assigned between bees. Once presentation took place, a positive response was evident by proboscis extension. A positive response was recorded as a '1' and a negative response as '0'. This examination was used to determine if Africanized bees or European bees are excited longer after the presentation of sucrose. This level of excitation could be a distinguishing factor due to the predetermined difference in aggression [17].

Results

Table 1 shows the comparison of percent positive response for the control and treatment groups. The analysis showed no significant differences between subspecies at any specific time interval and failed to reject the null hypothesis: no change in bee response over time. Probit regression was not performed because significant differences were not found at any specified time interval. With no significant trends or differences found, replicates of experiment one were not repeated to increase sample size.

Experiment Two: Appendage Mobility and Sting Viability

The second area of interest involved appendage mobility and viability of a sting after decapitation, which is possible due to the independent nervous system of the stinging apparatus [18,19,20]. Bees were captured, harnessed, and fed in the same manner as experiment one, but appendage mobility and sting viability were examined after decapitation. Two hundred fifty bees were collected, of which, 125 bees were European and the remainder Africanized. The 125 bees were subdivided into five groups of 25 bees each for each subspecies. These five groups differed in the amount of time (1 h, 8 h, 16 h, 24 h, and 36 h) that passed between decapitation and measurement of mobility and viability.

Table 1- Comparison of percent positive response for the central excitatory states between European (EHB) and Africanized (AHB) honey bees (*Apis mellifera*) stimulated by water only or sucrose followed by water (Stillwater, OK 2004)

	Water only				Sucrose-water			
ISI	**EHB**	**AHB**	**p-value**	**df**	**EHB**	**AHB**	**p-value**	**df**
1.5 seconds	64.00	48.00	0.254	1	92.00	72.00	0.065	1
60 seconds	40.00	40.00	1.000	1	52.00	48.00	0.777	1
120 seconds	48.00	68.00	0.152	1	80.00	52.00	0.036	1
240 seconds	40.00	56.00	0.257	1	68.00	56.00	0.382	1
360 seconds	60.00	72.00	0.370	1	76.00	64.00	0.354	1
480 seconds	44.00	40.00	0.774	1	60.00	64.00	0.770	1
600 seconds	48.00	52.00	0.777	1	64.00	60.00	0.770	1

Results

Analysis of initial data revealed no significant differences between European honey bees and Africanized honey bees at specified time intervals; however, significant trends for EHB mobility ($F_{3,4} = 50.27$, $p = 0.0058$), AHB mobility ($F_{3,4} = 233.32$, $p = 0.0006$), EHB viability ($F_{3,4} = 50.27$, $p = 0.0058$), and AHB viability ($F_{3,4} = 25.71$, $p = 0.0148$) were observed.

The evidence of significant trends provided a basis to perform replicates of this experiment to increase the sample size and precision of the estimates. Four replicates were performed for each subspecies, increasing the sample size from 25 bees to 125 bees per time interval for each subspecies. Figures 2 and 3 plot the number of positive responses (out of 125) recorded at each specific time interval.

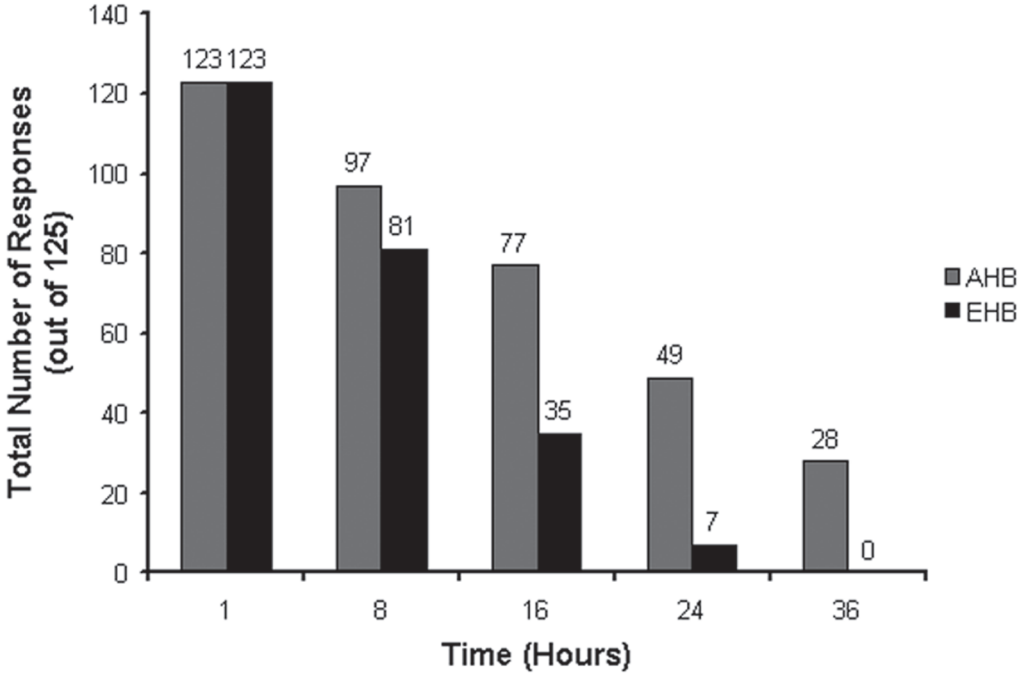

Figure 2- Comparison of appendage mobility after decapitation for Africanized (AHB) and European (EHB) honey bee, two subspecies of *Apis mellifera*

The response rates for both subspecies are equal, for appendage mobility ($\chi^2_1 = 46.56$, $p < 0.001$) and sting viability ($\chi^2_1 = 49.07$, $p < 0.001$). This showed that the data must be modeled individually for appendage mobility and sting viability over time for each subspecies. Figures 4 and 5 plot the probability for response over time for appendage mobility and sting viability, respectively. After plotting the data for each subspecies individually, a significant trend was found for European appendage mobility ($\chi^2_1 = 78.12$, $p < 0.001$), European sting viability ($\chi^2_1 = 67.31$, $p < 0.001$), Africanized appendage mobility ($\chi^2_1 = 37.77$, $p < 0.001$), and Africanized sting viability ($\chi^2_1 = 56.76$, $p < 0.001$).

With the presence of significant trends for appendage mobility and sting viability over time, each character state was analyzed to see at what point in time significant differences occurred. Table 2 shows the comparisons of probability of response for each character state by subspecies. A significant difference between subspecies for appendage mobility was found at and beyond the 8 hour time interval: 8 h ($\chi^2_1 = 4.99$, $p = 0.0254$), 16 h ($\chi^2_1 = 28.53$, $p < 0.0001$), 24 h ($\chi^2_1 = 40.59$, $p < 0.0001$), 36 h ($\chi^2_1 = 31.53$, $p < 0.0001$). A significant difference between subspecies for sting

Table 2- Comparison of percent positive response of appendage mobility and sting viability between European (EHB) and Africanized (AHB) honey bees (*Apis mellifera*) after decapitation (Stillwater, OK 2004)

	Mobility				Viability			
Time (hours)	**EHB**	**AHB**	**p-value**	**df**	**EHB**	**AHB**	**p-value**	**df**
1	98.40	98.40	1.000	1	98.40	93.60	0.0528	1
8	64.80	77.60	0.0254	1	63.20	70.40	0.2268	1
16	28.00	61.60	<0.0001	1	28.00	52.80	<0.0001	1
24	5.60	39.20	<0.0001	1	5.60	34.40	<0.0001	1
36	0.00	22.40	<0.0001	1	0.00	16.80	<0.0001	1

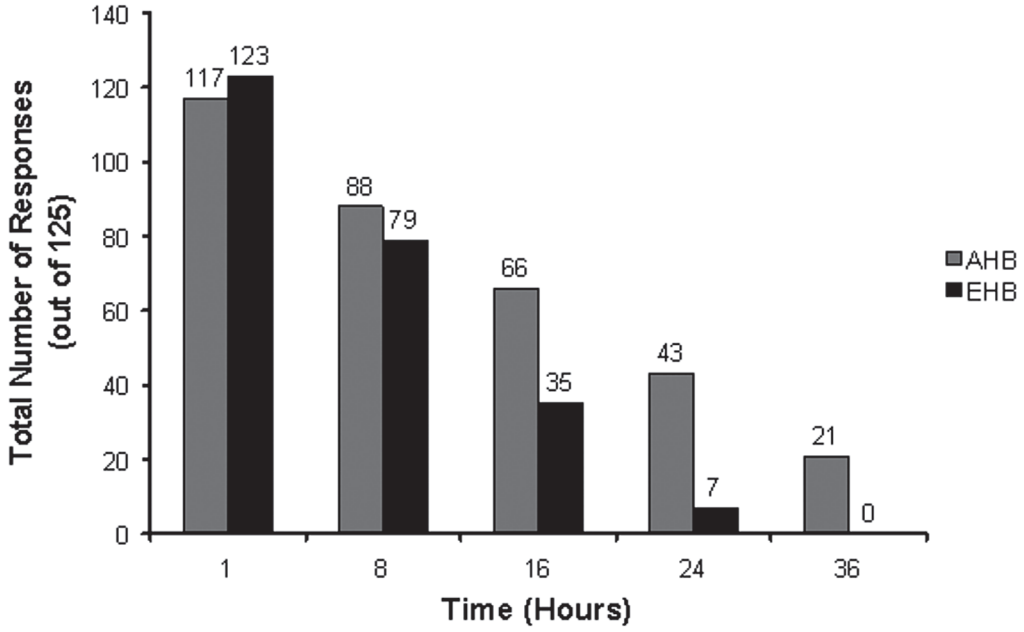

Figure 3- Comparison of sting viability after decapitation for Africanized (AHB) and European (EHB) honey bee, two subspecies of *Apis mellifera*

viability was also found, but at and beyond the 16 hour time interval: 16 h ($\chi^2_1 = 15.96$, $p < 0.0001$), 24 ($\chi^2_1 = 32.40$, $p < 0.0001$), 36 h ($\chi^2_1 = 22.93$, $p < 0.0001$). No European honey bees were observed to be mobile or to have a viable sting after 24 h; however 28 out of 125 Africanized honey bees were observed mobile and 21 out of 125 were observed viable at 36 hours after decapitation.

Discussion

Sting viability and appendage mobility can be used to distinguish between Africanized and European honey bees. Central excitatory state was not useful in distinguishing between subspecies. Africanized honey bees were mobile and viable for a longer period of time, thereby supporting our assumption of a higher predisposition to survival for Africanized honey bees.

Appendage mobility and sting viability are inexpensive techniques that can distinguish Africanized honey bees from European honey bees. These techniques do not require the expensive analytical equipment or training needed to perform currently used techniques. Mobility and viability analysis provide a way for the general public and the apicultural community to determine if their commercial European hives have been Africanized.

The ease of application of this technique and its relatively low cost make it suitable for anyone to use. Members

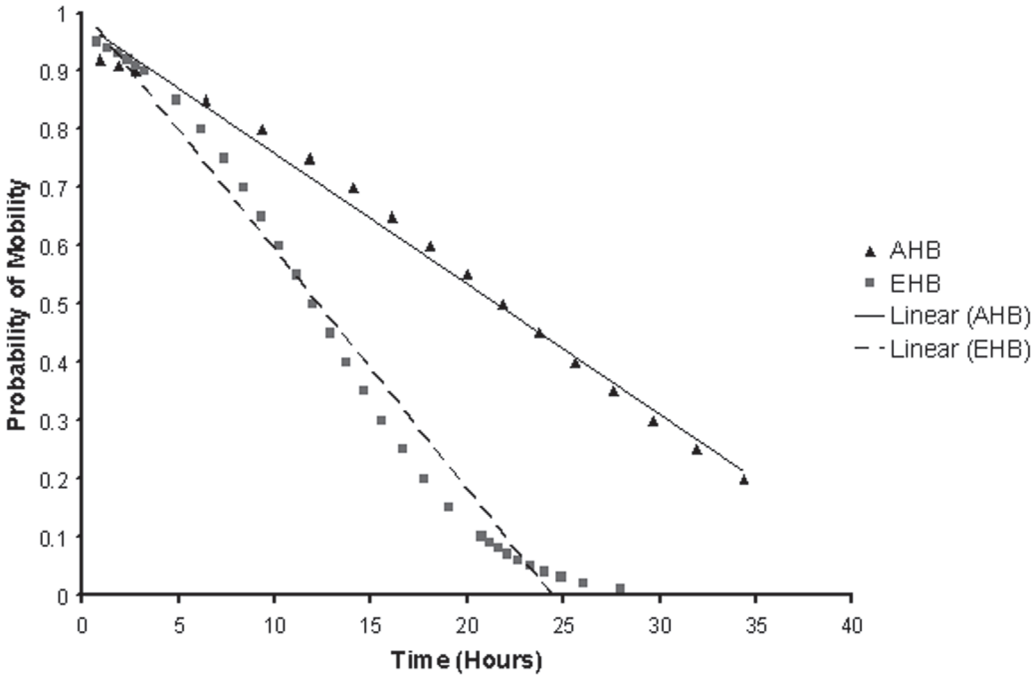

Figure 4- Effect of time on probability of appendage mobility in decapitated European (EHB) and Africanized honey bees (AHB), two subspecies of *Apis mellifera*

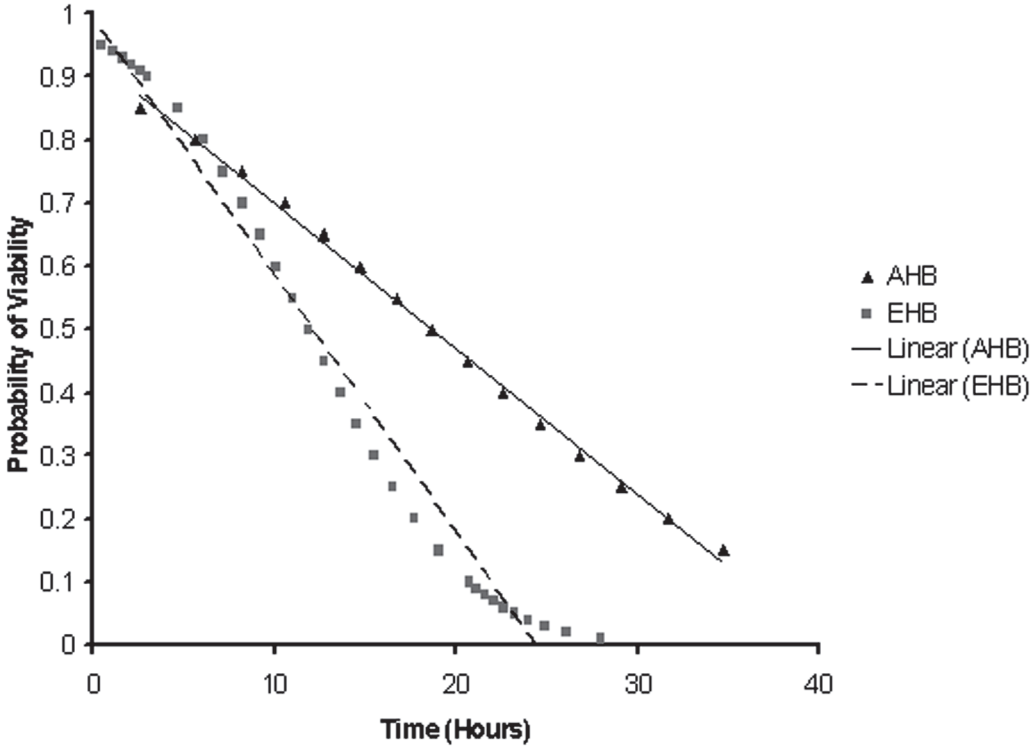

Figure 5- Effect of time on probability of sting viability in decapitated European (EHB) and Africanized honey bees (AHB), two subspecies of *Apis mellifera*

of the general public and the apicultural community can follow the procedures used in this study and have initial results in 36 h (Figure 6). A member of the public can apply these techniques to a sample size of 25 honey bees and achieve the same results. If a honey bee from a wild or commercial hive is mobile or has a viable sting 36 h after decapitation, it is Africanized. Interested parties may also examine a sample at 24 h. If the percent mobile or viable is greater than or equal to 30 percent, then the hive contains a high percentage of Africanized honey bees and should be tested further. These techniques provide an initial discriminatory test that will allow beekeepers to continually monitor the status of their hives without the use of highly advanced laboratories. If the preliminary test suggests the presence of Africanized honey bees, analytical tests can be initiated.

Our next step in refining these techniques is to apply them to more introgressed communities. This research analyzed the differences at the two extremes of the spectrum (African vs. European). Now research must be conducted to see to what degree of introgression these techniques are effective in distinguishing the two subspecies. Along with analyzing hives with greater degrees of introgression, narrowing the time span between 24 h and 36 h may be of interest to gain a more precise dividing line between the 2 subspecies.

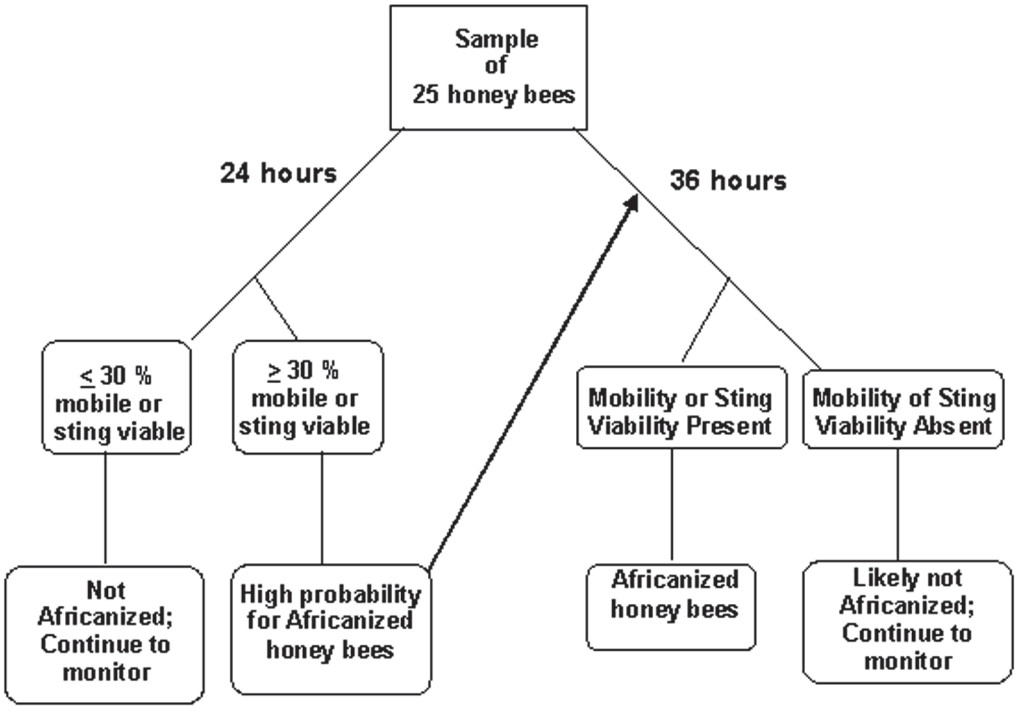

Figure 6- Steps for continuous monitoring of European honey bee (*Apis mellifera*) hives to prevent africanization

Uma maneira prática de distinguir abelhas africanizadas (*Apis mellifera L.*) de abelhas européias usando o estado excitatório central, a mobilidade dos membros e viabilidade do ferrão

Resumo

Uma chave para o entendimento da expansão da abelha africanizada (*Apis mellifera*) é distinguir esta abelha agressiva de sua equivalente européia. Técnicas de identificação atual têm um grau de sucesso, mas cada método tem seu próprio conjunto de problemas, tornando-se proibitivo sua adoção e uso em larga escala. Este estudo examinou aspectos do estado excitatório central, persistência de mobilidade de apêndices e viabilidade da ferroada após decapitação como ferramenta na distinção entre essas duas raças. O estado excitatório central não foi útil na distinção entre a abelha africanizada e a européia; todavia, a mobilidade de apêndices e a viabilidade do ferrão foram significativamente diferentes entre essas duas raças. A mobilidade de apêndices e a viabilidade do ferrão são técnicas úteis na distinção das duas raças e atenua os aspectos relacionados a gastos, aplicação e precisão.

Palavras-chave:
Condicionamento clássico.
Comportamento.
Abelha operária.
Abelha africanizada.
Abelha européia.

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