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Repertoire of Defensive Behavior in Africanized Honey Bees (Hymenoptera: Apidae): Variations in Defensive Standard and Influence of Visual Stimuli

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Abstract. The Africanized honey bees (AHB) are known by the high productivity and tolerance against pathogens and parasites such *Varroa destructor*. Besides these beneficial characteristics, the AHB are considered highly defensive and generally urges caution in management. However, little is known about the behavioral aspects of AHB in Brazilian beekeeping. In this context, our objectives were to evaluate the repertoire of defensive behavior (DB) in AHB emphasizing the relevance of environmental and visual stimuli, as well as the aggressiveness gradient among Brazilian colonies. The aspects related to defensive behavior were measured by Stort method with some adaptations. We found differences between colonies in the speed of first attack and attack intensity ($p<0.05\%$), although the DB patterns were not affected by temperature and period of evaluations. All attacks began in the black part of sphere target; however after the first sting both parts (black and white) were attacked. This fact indicates that even the visual clues have a relevant role in colony defense; chemical signs (alarm pheromones) maintain the target and act in attack increase. We also identified a great variation in defensive pattern among the studied colonies that was indeed expected due to high genetic variability present in AHB. The DB evaluation plays an important role in colony selection and genetic improvement based on beneficial characters such as high honey production and low aggressiveness during management.

Keywords: Aggressiveness; Apiculture; Apitoxin; Behavioral Responses.

Repertório do Comportamento Defensivo em *Apis mellifera* L. africanizada (Hymenoptera: Apidae): Variações nos Padrões Defensivos e Influência de Estímulos Visuais

Resumo. As abelhas africanizadas (AHB) são conhecidas por sua alta produtividade e tolerância a patógenos e parasitas como o ácaro *Varroa destructor*. Em contraste às características vantajosas, as AHB são consideradas altamente defensivas necessitando de certos cuidados em seu manejo. Entretanto, poucos estudos relatam características específicas sobre os padrões de comportamento de AHB na apicultura brasileira. Nesse contexto, o objetivo do trabalho foi avaliar o repertório do comportamento defensivo (DB) em AHB, identificando a importância de condições climáticas e estímulos visuais nesta característica, bem como gradiente de agressividade dentre as colônias. Os aspectos relacionados ao comportamento defensivo foram avaliados pelo método de Stort com adaptações. Foram encontradas diferenças entre as colônias em relação à velocidade do primeiro ataque e intensidade de ataque ($p<0.05\%$), por outro lado os padrões de DB não foram afetados pela variação de temperatura durante os períodos de avaliação. Todos os ataques se iniciaram pela parte preta da esfera alvo, contudo após o primeiro ataque ambos os lados (preto e branco) sofreram ataques. Tal fato indica que embora as pistas visuais possuam um papel relavente na defesa das colônias, sinais químicos (feromônios de alarme) atuam na manutenção e aumento do possível alvo. Pôde-se também identificar uma grande variação nos padrões defensivos dentre as colônias estudadas, fator esperado devido a grande variabilidade genética presente em AHB. Por sua vez, a avaliação do DB possui um importante papel na seleção e melhoramento de colônias visando à seleção de características benéficas como alta produtividade de mel e baixa agressividade durante o manejo.

Palavras-chave: Agressividade; Apicultura; Apitoxina; Respostas Comportamentais.

The Africanized Honey Bees (AHB) are known by the high productivity and tolerance against pathogens and parasites such as *Varroa destructor* Anderson & Trueman (Moretto *et al.* 1991; Pinto *et al.* 2011a, 2011b; Pinto *et al.* 2012). However, apart from this characteristic, this bee lineage is considered highly defensive with mass attack behavioral patterns (Guzmán-Novoa *et al.* 2004). Due the highly defensive behavior, the management urges caution and differs from that of European lineages requiring appropriate individual equipment and large bee smoker (Nascimento *et al.* 2005). Although considered aggressive the AHB demonstrate a great variation in defensive behavior (DB) that can be explained by its high genetic variability due these bees are results from cross breeding of the African honey bee *Apis mellifera scutellata* Linnaeus and European races (Guzmán-Novoa *et al.* 2004; Guzman-Novoa *et al.* 2005).

Acting together with genetic factors, the environmental variations (temperature and humidity) between regions also interacts in defensive behavior (Moritiz *et al.* 1987). In warmer regions such as the northeast of Brazil, the defensive behavior in AHB tends to be more intensified but evaluations in different seasons and regions must be carried to reinforce this aspect (Couto & Coutto 2002). Studies in honey bees relate the beginning of defensive behavior with visual stimuli by certain colors in combination with movements and specific odors (Roman & Gladysz 2009). On the other hand, little is known about the photo-sensitivity and behavioral answers of these insects on different wavelengths such as ultraviolet (UV) (Avarguès-Weber *et al.* 2012; Reser *et al.* 2012).

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As a way to mitigate aggressiveness and emphasize positive aspects like high production, selection breeding methods are shown to be promising in modern beekeeping. In this context, we evaluated the DB in Africanized honey bees highlighting the importance of visual stimuli among other factors in the defense of the colony.

MATERIAL AND METHODS

The experiments were conducted at the experimental apiary of the Universidade Federal de Viçosa, Minas Gerais, Brazil (-20° 45' 37.99", -42° 51' 55.50"). We used in the experiments three highly populated colonies (approx. 60,000 workers) maintained in Langstroth hives with food and brood combs.

The aggressiveness was measured by Stort method (1974) with some adaptations. We utilized a 3cm diameter sphere half coated with a black fabric and the other half coated with white fabric painted with UV dye. The evaluated parameters were: (a) occurrence time of the first sting in the sphere, (b) Number of stings in each color part of sphere and (c) Total number of stings during each application test (60 seconds). During each test, the behavioral alterations were observed and recorded to construct an ethogram of defensive behavior in AHB (Table 1).

The tests were performed three times per day (7:30h, 12h e 16h) in each colony during three consecutive days. The local temperature and relative humidity were also recorded in each test. To prevent the interference of concentration of alarm pheromone in materials, the spheres were not reused during the tests. To verify differences in defensive patterns between colonies, an analysis of variance (ANOVA) was performed, followed by Dunn test. In turn, the relations between environmental factors and DB were performed by Pearson Correlation Coefficient.

RESULTS

An ethogram of defensive behavior was generated by focal observations during the trials (Table 1). Seven events were observed and were allocated into two key-events ("Identification of invader" and "Attack"). Both evaluated aspects in defensive behavior (time for first sting and attack intensity) differed significantly between colonies ($p<0.05$). However, there were no correlations between climatic variables and the aspects studied in defensive behavior. Additionally, we also do not find effects summations over the evaluated days.

In all evaluations during the study, attack beginning (first sting) occurred in the black portion of sphere. The general attack preference for the black color was clear in all evaluations (Figure 1). Colony 01 (C1) attacked only the black side in all evaluations, whereas 76% of the C2 attacks were to the black side. C3 did not attack either sides in any test, although exhibited behavioral changes such as increase of wing beats during the trials.

DISCUSSION

Defensive patterns in Africanized honey bees. Social insects have developed elaborated defensive patterns; additionally the identification of possible invaders is an essential component in colony defense (Collins & Kubasek 1982). In *A. mellifera* the visual and chemical communication are utilized both in search of resources and in colony defense (Collins & Kubasek 1982; Gullan & Cranston 2010). In our work, the black color associated with

Table 1. Ethogram of defensive behavior in Africanized honey bees: The pre-attack and attack components observed and the mean time of each behavior.

Key events	Time (sec.)	Description of Behavior
Identification of invader	0.1±1	Bees located at the entrance of the colony begin to flap their wings rapidly
	12±10	The 1st bees begin to fly towards the target
	16±13	The bees begin to clash against target
Attack	25±20	The first sting occurs, followed by the release of the stinger with venom sac on target
	25±20	Bees that have stung continue flying and attacking the target
	26±22	The target takes other stings near the 1rst
	26±22	The intensification of the attack occurs

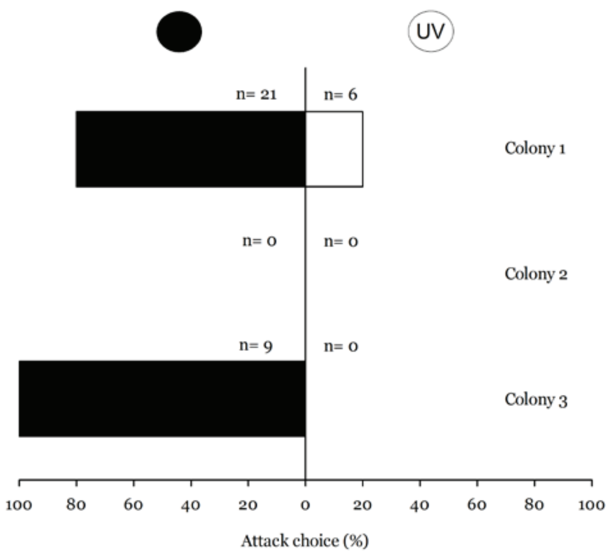

Figure 1. Percentage response of defensive behavior in accordance with the target color pattern. n= Total number of stings per color in all trials.

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movements near the colony promoted the first attack responses. However the portion coated with white fabric painted with UV dye was also attacked posteriorly, even with a lower intensity than the black part indicating the pheromone importance in colony defense.

The innate defensive patterns against certain textures and dark colors can be an elaborate instinctive answer due the selective pressures of mammalian predators (Free 1961). Although, due to the high capacity of chemical communication, invaders without such features (dark hairs e.g.) are identified by their odors and after the first sting, alarm pheromones increase the target area (Collins & Kubasek 1982; Visscher *et al.* 1995; Kastberger *et al.* 2009; Roman & Gladysz 2009).

The visual and chemical stimuli also interact in avoiding behaviors (Shorter & Rueppell 2012). Typical behaviors such as intensive wing-beats and hitting the target before the sting could possibly be a warning behavior by the individual, thus avoiding the suicidal DB (Tan *et al.* 2012). We observed these behaviors before and after the first stings. In our point-of-view this fact shows a great role in colony defense. The continuous attack of bees that have stung can enhance the visualization of the target, furthermore this behavior acts like an attempt to intimidate and expel intruders (Cunard & Breed 1998; Shorter & Rueppell 2012).

Genetic variability *vs.* DB. AHB present a great genetic variability due the hybrid character of this race originated from cross breeding between European and African bees (Collet *at al.* 2006). In this context, it is very possible to observe great variations in defensive behaviors between colonies in the same region (Breed *et al.* 2004). We could observe this variation comparing the behavioral differences between colonies regardless of climatic factors and evaluation period. Similar results were also found with AHB in Mossoró, Northeast of Brazil where expressive changes were observed in response speed and intensity of attacks among colonies evaluated (Nascimento *et al.* 2008).

Regardless the behavioral variability, AHB normally present faster and higher defensive pattern compared to European races. In a study using Africanized / European co-fostered colonies, 81% of the initial attacks were performed by Africanized individuals (Guzmán-Novoa *et al.* 2004). However, even with the role of regulatory genes, the biotic and abiotic factors such as physiological condition of the colony and environmental changes are also involved in behavioral changes (Alaux *et al.* 2009). Although we found no relationship between temperature and DB, more intensive temperature changes may affect the DB. In northeast region, AHB colonies have varied the average number of stings from 16.7 to 22.7 in accord to the daily fluctuation of temperature (Nascimento *et al.* 2005).

Assessments in different periods with different climatic conditions can gauge the importance of this character. However, the colony population change due to a longer period of time as food availability may also affect the comparisons. Apparently several biotic and abiotic factors interact in defense of the colony, which may be considered by plasticity of the DB in *A. mellifera*. In our study, we identified a great variation between colonies in the repertoire of defensive behavior even being kept under the same conditions. Visual stimuli including color and movement were important components in DB of AHB. However, inter / intraspecific chemical stimuli favor the identification of possible invaders and allow joint defensive actions in favor of the colony.

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