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Review

Results and impact of research on honeybee genetics and breeding conducted by INIFAP in Mexico

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Abstract:

In Mexico, beekeeping is an activity of economic, social and ecological importance that faces various problems; two of the most important problems are the high defensive behavior of honeybee colonies (*Apis mellifera* L.) caused by the africanization and varroosis caused by the mite *Varroa destructor*. The high defensive behavior of the colonies has made beekeeping more complex and less profitable. *Varroa destructor* affects honey production and is a factor that has been associated with honeybee colony losses worldwide. To address these problems, INIFAP conducts research on honeybee genetics and breeding. The objective of this article was to make a review of the research conducted at INIFAP in honeybee genetics that has created scientific knowledge about the genetic, genomic, and epigenetic factors that regulate the expression of honeybee defensive behavior, guarding behavior, stinging behavior, grooming behavior and hygienic behavior. To review the results of research conducted by INIFAP in honeybee breeding to reduce the defensive behavior of honeybee colonies, this research has created methods to evaluate and to select this trait, and has generated honeybee lines with low defensive behavior, from which queens has been transferred to beekeepers. As well as to review the work conducted by INIFAP to preserve genetic material of European origins that has led to the establishment of a honeybee germplasm bank.

Key words: Honey bees, Genetics, Breeding, Defensive behavior, *Varroa destructor*, Grooming behavior.

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Introduction

Importance of beekeeping in Mexico

Mexico is one of the leading producers and exporters of honey globally; it currently ranks as the tenth producer of honey and the fifth exporter worldwide(1). From 2010 to 2019, the average annual honey production was 58,094 t, with an average annual yield of 29.7 kg per hive(2). During this same period, 34,745 t of honey per year were exported, representing 60% of the production(1). The honey produced in Mexico has a high demand in the international market due to its quality traits. The commercial value of honey production in Mexico for 2010-2019 was 2,924 million pesos per year(1).

In Mexico, there are 2’157,866 bee colonies, which belong to approximately 43,000 beekeepers(2). Approximately 70 % of beekeepers are small producers, for whom the sale of honey and wax represents an important part of their income. Around 60 % of the colonies in Mexico belong to this type of beekeepers, who have a low degree of technification and manage 40 hives on average. The remaining colonies (40%) belong to production units of various sizes and degrees of technification(3).

In addition to the production of honey, wax, pollen, royal jelly, and propolis, the pollination carried out by honeybees is essential for maintaining the balance of the ecosystems and for food production. Honeybees partially or completely pollinate approximately 70 % of the plants cultivated for human consumption. Thus, humans heavily depend on honeybees for feeding purposes. In Mexico, the value of the pollination carried out by honeybees in cultivated plants is 20 times greater than the value of honey production(4). The estimated value of the pollination of cultivated plants from 2010 to 2019 was 58,480 million pesos per year.

Mexico is divided into five beekeeping regions: North, Plateau, Pacific Coast, Gulf, and Yucatan Peninsula. These regions are classified based on their climate and vegetation(5). The Yucatan Peninsula, Plateau, and Pacific Coast regions are the major contributors to honey production. These regions produce 36 %, 25 %, and 22 % of the national honey production, respectively, equivalent to 32 %, 27 %, and 24 % of the economic production value. The North and Gulf regions produce 9 % and 8 % of the national honey production, representing 9 % and 8 % of the total economic value, respectively(2).

Main beekeeping problems in Mexico in which INIFAP has conducted research

The highly defensive behavior of honeybee colonies caused by Africanization and varroosis caused by *Varroa destructor* are the two main problems that beekeeping faces in Mexico, for both INIFAP has conducted research in the fields of genetics and breeding to reduce the impact of these problems.

The Africanization of honeybee populations that has occurred during the last 60 years in the American continent is considered one of the cases with highest impact of an invasive species in history. Africanized honeybees originated in Brazil from the cross of European (*A. mellifera mellifera*, *A. mellifera ligustica*, and *A. mellifera carnica*) and African honeybees (*A. mellifera scutellata*), which were introduced into this country in 1956(6).

Africanized honeybees have short brood development stages(7) and high reproductive rates, which translates into high swarm production and facilitates the dispersion of these honeybees throughout South America and Central America, reaching Mexico in 1986(8) and the United States of America in 1990(9).

Africanization had drastic consequences for beekeeping and even public health in many countries(10). Since 1986, when Africanized honeybees reached the country, Mexican beekeeping has suffered important changes due to Africanization(11). Compared to European honeybees, these honeybees are more defensive, show a greater tendency to swarm and evade, and produce less honey(12-15).

The highly defensive behavior of Africanized bee colonies has complicated their management; this has decreased profitability due to the increased production costs derived from the practices implemented by beekeepers to manage this type of bee(11).

Africanized honeybees are distributed throughout the country, and the degree of Africanization of bee populations managed by beekeepers is high(16). A study carried out in Yucatan indicated that 61 % of the colonies managed by beekeepers and 87 % of the wild colonies are Africanized(17). Meanwhile, a different study reported that 100 % of the analyzed colonies were Africanized[16]. In Mexico State, a study estimated that 37 % of the colonies managed by beekeepers were Africanized, and 70 % had some degree of Africanization[18]. In Morelos, a study indicated that 65 % of the colonies showed some degree of Africanization[19].

Varroosis represents a severe threat to the survival of honeybees and honey production both in Mexico and the world. A study in Mexico indicated that colonies with infestation levels of 6% produced on average 65 % less honey than parasite-free colonies[20]. A different study reported that the production of honey decreased by 52.8 g per infestation percentage unit[21]. Furthermore, this parasite has been associated with colony collapse disorder (CCD). In recent years, this phenomenon has been responsible for the loss of bee colonies in Mexico[22,23] and other parts of the world[24-27].

Varroosis is controlled with synthetic chemical acaricides and organic chemical products. None of the products are 100 % effective against this mite. The synthetic acaricides used in Mexico are flumethrin and fluvalinate, which have a reported efficiency of 98 %. However, several studies in Mexico and in other countries have reported mites resistant to these products; acaricides have an efficiency lower than 50 % against resistant *V. destructor*[28,29]. Furthermore, these products can leave chemical residues on the honey and wax, affecting both honeybees and humans[30-34]. The organic chemicals most used in Mexico for mite control are thymol, oxalic acid, and formic acid. Although these products do not generate resistance, their efficacy is 93 % or less[35].

Varroa destructor was first detected in Mexico in 1992[36], and this mite is now distributed throughout the country. A study carried out in Zacatecas reported that the prevalence of varroosis is 89 %, with an average level of infestation of 4.85 %[37]. In Morelos, a prevalence and average infestation level of 80% and 4.76 %, respectively, were reported[38]. Jalisco has a prevalence of 88% and an infestation level of 5.2 %[39]. Varroosis is 63 % prevalent in Yucatan, with an infestation level of 1.70 %[40].

This article aims to review the results obtained from research studies conducted in Mexico and outside the country by INIFAP or with the participation of INIFAP researchers. This review includes research in genetics and breeding of honeybees related to their defensive behavior and resistance to varroosis.

Results of studies conducted on honeybee defensive behavior genetics

The studies that have been carried out in Mexico to know/understand the genetic mechanisms that regulate the defensive behavior expression in honeybees have allowed determining that colony defensive behavior, which is measured by the number of stings left by the honeybees of the same colony on a black suede flag waved in front of the beehive for a specific period of time, is regulated by dominant genetic effects; since, when compared between European, Africanized and hybrid genetic groups, defensive behavior has significant differences between European and Africanized groups, and defensive behavior of colonies belonging to the hybrid group is as high as the one of the Africanized group. In addition, these studies have allowed determining that interactions between honeybees belonging to the three genetic groups within the same colony have an influence on the defensive behavior of that colony. This means that if European genotype honeybees interact with honeybees of the African or hybrid groups within the same colony, the defensive behavior of such colony will be as high as the defensive behavior of a colony formed only by hybrid of Africanized honeybees[41,42,43].

Mexico was the first country to identify regions within the bee genome that regulate the expression of the defensive behavior of the colonies. In this study, five Quantitative Trait Loci (QTL) were linked to the expression of defensive behavior using a population of 172 colonies formed by a single backcross-derived family of worker bees obtained following a crossbreeding scheme from Africanized and European honeybees[14]. The effect of three of the five QTL on the defensive behavior of honeybees was confirmed by two independent studies in Africanized[43] and European honeybees[44].

The defensive response of a colony involves two behaviors carried out by the colony's worker honeybees at the individual level, the guard and stinging behaviors[45-51]. In Mexico, some studies evaluated the relationship between the guard and stinging behavior during the defensive response of a bee colony. Their results indicate that the guarding bees participate in the defensive response of the colony; thus, their presence, number, and proportion of bees that respond by stinging influence the intensity of the colony's defensive response[44,50,51].

Furthermore, researchers in Mexico have evaluated the genetic mechanisms that regulate the expression of the guarding and stinging behaviors at an individual level. These studies are essential to understanding how behaviors that are genetically regulated at an individual level can influence the phenotype of the entire colony.

Additionally, previous studies have observed that, at an individual level, the stinging behavior (measured as the time it takes for a bee to sting a piece of black suede after receiving a constant electrical stimulus) of Africanized honeybees is significantly higher than in European honeybees. Thus, the response of Africanized honeybees is faster than that of European honeybees. Furthermore, one of these studies reported that the stinging behavior of bees with guarding behavior at the hive's entrance and bees that respond by trying to sting an intruder was superior compared to the bees that carry out other duties within the colony; this was observed in both Africanized and European colonies[49].

Arechavaleta-Velasco *et al*(44) determined that three of the QTL previously associated with the defensive behavior of bees at a colony level(14) affect the stinging behavior of individual honeybees. However, Shorter *et al*(52) identified two new QTL for this trait, from which four candidate genes could be identified for the expression of the stinging behavior of honeybees.

As for the guarding behavior, studies have identified effects of genetic origin for the number of bees that perform guarding behavior in a honeybee colony(44).

Arechavaleta-Velasco and Hunt(53) identified seven Binary Trait Loci (BTL) associated with the expression of guarding behavior in worker bees using two reciprocal backcross-derived bee colonies generated from European lines with high and low defensive behavior. A different study determined that one of the QTL previously associated with the defensive behavior of bees at a colony level(14) affects the guarding behavior of individual bees(44). Meanwhile, Shorter *et al*(52) identified one new QTL for this trait from which three candidate genes could be identified for the expression of the guarding behavior of bees.

Guzmán-Novoa *et al*(54) observed that the defensive behavior of hybrid colonies derived from reciprocal crosses between Africanized and European bees is different. The hybrid colonies derived from the cross between European queens and Africanized drones are significantly more defensive than the hybrid colonies derived from the cross between Africanized queens and European drones. The phenotypic differences between reciprocal hybrid groups are an indicator of epigenetic effects(55,56). Thus, these results suggest that the increased defensive behavior of the colonies whose paternal origin is Africanized is due to the epigenetic effects generated by an imprinting process of paternal origin.

A study carried out by Kocher *et al*(57) analyzed the transcriptome of different tissues of bees with reciprocal hybrid genotypes obtained from a crossbreeding scheme between Africanized and European bees. These researchers reported that the selective expression of genes by their paternal origin is one of the mechanisms by which epigenetic effects occur. The selective expression of genes by their parental origin occurs when the expression level depends on whether an allele is inherited through the mother or father.

Gibson *et al*(58) found differences in the stinging behavior of bees at an individual level between reciprocal hybrid genotypes that originate from crosses between European and Africanized bees. Hybrid bees whose paternal origin was Africanized responded to an electrical stimulus by stinging a piece of black suede much faster than the hybrid bees with a European paternal origin. These observations indicate that, as reported for the defensive behavior, epigenetic effects exist due to an imprinting process of paternal origin.

The transcriptome of the genes localized in the genomic regions that correspond to the QTL associated with the defensive behavior was analyzed to evaluate if the epigenetic effects detected in the stinging behavior of individual genes(58) result from the selective gene expression due to their parental origin(14,43,44,52,59). This analysis was carried out in bees, for which the stinging behavior had been individually evaluated. The results indicated that this mechanism is involved in the regulation of gene expression in hybrid bees with European maternal origin and Africanized paternal origin. This was apparently due to disturbances in the signaling pathways between nuclear and mitochondrial genes that modulate brain metabolism and defensive behavior in bees(58).

Results of studies and breeding programs to reduce the defensive behavior of honeybees

Guzmán-Novoa and Page(59) reported the results of a breeding program that started in November 1991 and concluded in 1996. This program was implemented for four generations of selection in a commercial population of approximately 3,000 bee colonies to improve honey production and reduce the defensive behavior of the population. This study demonstrated that it is possible to genetically improve bee populations in Africanized zones without instrumental insemination of honey-bee queens.

The breeding program consisted of conducting mass selection in stages, considering honey production, defensive behavior, laying pattern of bees, and the average length of the forewing of the worker bees in the colony. The results of this program showed that it was possible to maintain honey production, reduce the defensive behavior of the colonies, and increase the average length of the forewing of the worker bees in the population under selection despite the process of Africanization of bees that was occurring at the time in the region of study. These results indicate that the defensive behavior of the population decreases when the relative frequency of colonies with European morphotype and haplotype increases due to the selection process.

In 1996, INIFAP started a breeding program to generate lines of bees selected for high honey production and low defensive behavior. The breeding program was developed in Mexico's State; approximately 500 bee colonies were formed with colonies from INIFAP and cooperating beekeepers. The traits included in the program as selection criteria were: honey production, defensive behavior, the average length of the forewing of the worker bees in the colony, colony morphotype (European, hybrid, or Africanized), and colony haplotype (European or African)(60).

In this breeding program, three lines of bees were generated. In various studies, honey production, defensive behavior, the average length of the forewing of worker bees, morphotype distribution (European, Hybrid, and Africanized), haplotype distribution (European and African), and the genetic variability of the mitochondrial DNA have been evaluated and compared to European and Africanized populations outside breeding programs[(61-65)].

As for honey production, in two different studies, the genetically improved lines of bees produced significantly more honey than the bees that did not belong to a breeding program[(61,63)]. In one of the studies, INIFAP lines produced on average 34.5% more honey than non-selected Africanized bees[(61)]. In the second study, bees produced on average 27.5 and 40.3 % more honey than non-selected European and Africanized bees, respectively[(63)].

The defensive behavior was measured by counting the number of bee stingers deposited on a black suede flag waved in front of the hive for a specified time. Improved lines were on average 57.4 % less defensive than the non-selected Africanized bees and 44.3 % more defensive than the non-selected European bees[(63)].

Regarding the average length of the forewing of worker bees, a study evaluated two lines of INIFAP bees, one of these lines had longer (9.07 and 9.06 mm) forewings than European (9.03 mm) and Africanized (8.90 mm) bees. The forewing length of the second line was similar (9.02 mm) to that of non-selected European bees, and both groups had longer forewings than non-selected Africanized bees[(63)]. Another study observed that the improved lines of bees had longer forewings (9.05, 9.04, and 9.03 mm) than the non-selected Africanized bees (8.98 mm)[(65)].

Two studies evaluated the morphotype distribution in different colonies. They found that the frequency of bee colonies with European, Hybrid, or Africanized morphotypes in selected lines was significantly different from that observed in the populations of non-selected bees[(62,65)]. One study observed that in the populations of selected bees, the average relative frequencies of colonies with European, Hybrid, and Africanized morphotypes were 0.47, 0.35, and 0.18, respectively. In the population of non-selected colonies, the relative frequency of the European, Hybrid, and Africanized morphotypes were 0.17, 0.43, and 0.40, respectively[(62)]. In a different study, the population of three INIFAP lines showed average relative frequencies of 0.37, 0.42, and 0.21 for the European, Hybrid, and Africanized morphotypes, respectively. The frequencies for the populations of non-selected bees were 0.17, 0.43, and 0.41[(65)].

For the haplotype distribution, the average relative frequencies of the population of selected lines with European or African haplotypes were significantly different from that of the non-selected bee populations. The European and African haplotype frequency was 0.93 and 0.07, respectively, in the population of INIFAP bees, and in the population of non-selected bees, the frequencies were 0.34 and 0.66, respectively[(62)]. Finally, regarding the genetic variability of the mitochondrial DNA, estimated by the Shannon Index, the lines of selected bees showed lower variability (IS=0.12) than non-selected populations (IS=0.41); this resulted from the selections process to which bees were subjected[64].

Improved lines have been used to develop projects in which INIFAP has transferred the improved genetic material to beekeeper groups in Mexico's State, Hidalgo, Queretaro, and Morelos. These projects have provided 10,000 royal cells, 3,000 queens for free fertilization, and 500 instrumentally inseminated bees as breeding stock. From this genetic material, beekeepers have produced at least 20,000 bees for free fertilization[66].

Results of the studies conducted to preserve European honeybee germplasm

In 2004, INIFAP established a Germplasm Bank aimed to preserve the genetic material of European bees[67]. This Bank is in the National Center for Disciplinary Research in Animal Physiology and Genetics. This bank includes a population of 100 bee colonies, which are handled following a closed population scheme with instrumental insemination of queen bees[68,69]. This approach genetically isolates the population by having total control over mattings; only queen bees and drones from the germplasm are used for the crossings required to produce the queens for the annual queen replacement that has to be carried out in the population.

The germplasm is characterized in morphometric, molecular, and behavioral terms. Evaluations of the bee colonies are carried out every year to ensure that the genetic material kept in the bank has European characteristics. These genetic analyses use molecular markers in the mitochondrial DNA that classify bee haplotypes into European or African[70]. The length of the forewing of bees is determined through morphometric analyses, which allow classifying colonies into European, Hybrid, or Africanized[71]. The defensive behavior of colonies is evaluated by qualitatively determining how much the bees of a colony run on the honeycomb, fly over the hive, and collide and sting the beekeeper during their routine check[60]. Finally, the tendency of colonies to swarm and evade is also evaluated.

INIFAP's bee germplasm bank has been preserved for 17 generations, and each generation corresponds to an annual beekeeping cycle. The genetic material preserved in the bank has been used to develop research projects and generate, validate, and transfer improved genetic material[66].

Results of studies conducted on the genetics of honeybee resistance to varroasis

In Mexico, studies have evaluated the differences in susceptibility to *Varroa destructor* of Africanized and European bees[72,73]. Guzmán-Novoa *et al*[72] compared the susceptibility to *Varroa destructor* of adult bees and broods with Africanized, European, and two reciprocal hybrid genotypes. Their results indicate that the Africanized and two hybrid genotypes in adult bees were equally susceptible but less susceptible than the European genotype. As for the broods, the Africanized genotype was the least susceptible, followed by the European and hybrid genotypes originated from a European mother and an Africanized father; the hybrid originated from an Africanized mother and a European father was the most susceptible. This same study also evaluated the effect of these genotypes on the reproductive capacity of the mite and found similar capacities in European and Africanized genotypes; the highest reproductive capacities were observed in both hybrid genotypes.

Guzmán-Nova *et al*[73] analyzed the results of various studies carried out in Mexico to determine if the susceptibility to *Varroa destructor* was different between Africanized and European bees. These results indicate that the susceptibility or resistance to the mite does not depend on the genetic group as both groups show variability in their susceptibility to *Varroa destructor*. Furthermore, these studies found that the environment and the interaction between the genotype and the environment play an important role in the infestation levels of bee colonies.

The resistance to *Varroa destructor* depends on the expression of resistance mechanisms against this mite. These mechanisms include the grooming behavior, the hygienic behavior, the differential attraction of the worker brood and the adult worker bees to the mite, a shorter brood development period after capping, and factors affecting the fertility and reproduction of the mite[73-78].

Researchers in Mexico carried out one of the two studies that measured the relative contribution of each one of these mechanisms of resistance to *Varroa destructor*[75,79]. Arechavaleta-Velasco and Guzmán-Novoa[75] determined that, in Mexico, there is variability of genetic origin in the resistance of bee colonies to *Varroa destructor* and that this resistance is not related to one line or genetic great in particular. In this study, they evaluated how the grooming and hygienic behaviors of bees, the differential attraction of *Varroa destructor* to the brood, and the effect of the brood on the reproductive capacity of the mite contribute to the resistance of bee colonies to the population growth of this mite. This study indicates that grooming behavior is the primary mechanism that colonies use to resist the population growth of *Varroa destructor*. Furthermore, susceptible and resistant colonies showed differences in their hygienic behavior; however, the contribution to resistance of this behavior was not clear. On the other hand, Harbo and

Harris[79] identified the hygienic behavior against *Varroa destructor* as the primary mechanism of resistance.

Arechavaleta-Velasco *et al*[80] developed a study to identify the regions within the bee genome that regulate the expression of their grooming behavior. This study associated this grooming behavior to a quantitative trait locus (QTL), *groom*-1, located in chromosome 5 of the bee genome. Twenty-seven genes were identified within the region that corresponded to the 95 % confidence interval for the location of the QTL and reported in the Honeybee genome database; one of these genes was *neurexin-1* (*AmNrx1*). This gene's orthologs are associated with autism and schizophrenia in humans, synapses formation and associative learning in *Drosophila* and *Aplysia*, and grooming behavior in mice. The grooming behavior of *Neurexin-1-alpha* knockout mice is higher compared to wild-type mice. The fact that *neurexin*-1 influences grooming behavior in mammals and bees is evidence of the effect of this gene on the expression of this trait. The effect of the QTL *groom-1* and *neurexin-1* (*AmNrx1*) on the expression of the grooming behavior was confirmed using a bee population different to the one in which the effect of QTL and the gene was first identified[81].

As for the hygienic behavior, a study carried out at the level of individual bees identified seven BTL associated with the expression of this trait[82]. This number of BTL was similar to the number of QTL detected in the study carried out by Lapidge *et al*[83] to identify the genomic regions for this trait at the phenotypic level of the entire colony.

Impacts

The research developed in INIFAP regarding the genetics of the defensive behavior of honeybees and the mechanisms of resistance of the honeybees to *Varroa destructor* has had a substantial scientific impact worldwide. These studies have contributed to understanding the genetic, genomic, and epigenetic mechanisms that regulate the expression of the defensive behavior of colonies, the guarding and stinging behaviors of individual bees, and the grooming and hygienic behaviors.

As for reducing the defensive behavior of bee colonies, these studies have had a significant scientific and technological impact by generating methods for the evaluation, selection, and breeding for this trait. Honeybee lines with low defensive behavior were obtained by applying the knowledge provided by these studies. From these lines, 500 breeding stock queens and at least 33,000 queens of free fertilization have been transferred to producers, representing a significant social and production impact.

The studies developed by INIFAP to establish a Honeybee Germplasm Bank have an important ecologic and technological impact. This bank is the only nucleus of conservation of germplasm of European origin in Mexico. There is a high risk of losing the germplasm of European origin because of the high levels of Africanization in Mexico's honeybee populations; therefore, it is essential to preserve the honeybees maintained in this bank as a vital genetic resource for beekeeping.

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