Journal of Invertebrate Pathology 209 (2025) 108276

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Occurrence and allele frequencies of genetic variants associated with *Varroa* drone brood resistance (DBR) in African *Apis mellifera* subspecies

Haftom Gebremedhn [a,b,*], Regis Lefebre [a], Moses Chemurot [c], Felicien Amakpe [d], Bezabeh Amssalu [e], Lina De Smet [a], Dirk C. de Graaf [a]

[a] Laboratory of Molecular Entomology and Bee Pathology (L-MEB), Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium
[b] Mekelle Bee Research and Training Center, Tigray Agricultural Research Institute, Mekelle, Ethiopia
[c] National Agricultural Research Organization (NARO), National Livestock Resources Research Institute (NaLIRRI), P.O. Box 5704, Kampala, Uganda
[d] Direction Générale des Eaux, Forêts et Chasse: Centre Béninois de la Recherche Scientifique et de l'Innovation. BP 388, Abomey, Benin
[e] Holeta Bee Research Center, Holeta, Ethiopia

ARTICLE INFO

Keywords:
African honeybee subspecies
Genetic markers
SNPs
SMR
Varroa

ABSTRACT

The ectoparasite *Varroa destructor* is a major contributor to the global decline of honeybee colonies (*Apis mellifera*), especially in the Northern Hemisphere. However, *Varroa*-resistant honeybee populations have been reported in various regions around the globe, including Europe and Africa. This resistance is primarily attributed to the trait known as Suppressed Mite Reproduction (SMR), which significantly reduces the reproductive success of *Varroa* mites within these colonies. Although this trait is still poorly understood, several efforts have been made to unravel the genetic basis of SMR. For example, a study in Belgium determined eight genetic variants in the honeybee genome that are associated with the infertility of mites in drone brood (Drone Brood Resistance or DBR). As these eight variants were found and validated in subpopulations of European subspecies only, there is limited knowledge about the occurrence of these markers in African honeybees. Hence, this study was designed to determine the allele frequencies of these eight genetic variants in African honeybee populations. More specifically, we used qPCR assays with dual-labeled probes to analyze bee samples collected from Benin, Ethiopia, and Uganda. Our results showed the presence of seven of the eight variants in African *Apis mellifera* subspecies, which may contribute to their innate resistance against the *Varroa* mite. Moreover, we found significant differences in allele frequencies among the three sampled African bee populations, suggesting the presence of genetic diversity within these populations, potentially altering their resistance to *Varroa*. This study revealed similar allele frequencies between African honeybees and bee samples from the European *iberiensis*-subspecies (A lineage), while Ethiopian bees showed distinct distributions, indicative of a unique lineage. Overall, the occurrence of most DBR-associated genetic variants in African honeybees opens research opportunities to elucidate the predictive properties and potential of these genetic variants in the African continent by examining genotype-phenotype associations.

1. Introduction

The contribution of honeybees to biodiversity conservation (Al-Ghamdi et al., 2017; Bradbear, 2009), ecosystem protection (Sabbahi, 2022) and the improvement of the commercial value of agricultural crops (Calderone, 2012; Garratt et al., 2014; Klein et al., 2007) is significant. However, despite their ecological and economic importance, honey bee populations are facing substantial declines, especially in the Northern Hemisphere (Gray et al., 2019; Smith et al., 2014; VanEngelsdorp et al., 2008; Yalçınkaya & Keskin, 2010). This phenomenon may disrupt ecosystems, threaten future food and nutrition security and can lead to a severe pollination crisis. Several biotic and abiotic stressors associated with managed honey bee colony losses have been identified (Neov et al., 2019), with the ectoparasite *Varroa destructor* being the most significant biotic factor (Gray et al., 2019; VanEngelsdorp et al., 2008, 2012).

Varroa feeds on the fat tissues and hemolymph of pupae and adult bees, causing physical damage (Ramsey et al., 2019) and suppressing the

* Corresponding author.
E-mail address: haftush@yahoo.com (H. Gebremedhn).

https://doi.org/10.1016/j.jip.2025.108276
Received 4 November 2024; Received in revised form 14 January 2025; Accepted 25 January 2025
Available online 27 January 2025

honeybees' immune response (DeGrandi-Hoffman & Chen, 2015), which increases vulnerability to viral infections (Barroso-Arévalo et al., 2019; Shen et al., 2005). It also acts as an effective vector for numerous viruses such as the deformed wing virus (DWV) (Bowen-Walker et al., 1999; Brutscher et al., 2016; Posada-florez et al., 2019) , leading to increased viral loads within the colony (Martin et al., 2012). The synergistic relationship between viruses such as DWV and the ectoparasite *V. destructor* (Bowen-Walker et al., 1999) has been identified as a major cause of colony losses worldwide, along with challenging climatic and environmental conditions (Benaets et al., 2017; Currie et al., 2010; Kevill et al., 2019; Martin et al., 2012; VanEngelsdorp et al., 2012). Consequently, most beekeepers in the Northern Hemisphere have been using acaricides to control *Varroa* infestations in their colonies (Brodschneider et al., 2023). However, the continued use of synthetic acaricides has led to the development of mite resistance (Rinderer et al., 2010; Sammataro et al., 2005) and contamination of hive products (Büchler et al., 2010; Rinderer et al., 2010). Moreover, these treatments only work effectively on susceptible mites and require multiple intensive applications, which in their turn disturb the bees' natural harmony with the ecosystem. Therefore, there is an urgent need to use alternative non-chemical and non-invasive control methods for the mite's burden, including the use of mite-resistant honey bee lines (Brodschneider et al., 2023; Büchler et al., 2010; Rinderer et al., 2010).

Multiple *Varroa*-resistant honey bee populations that survive without mite treatments have been reported in different parts of Europe, such as Norway (Oddie et al., 2017), Avignon (France) (Le Conte et al., 2007), Gotland (Sweden) (Fries et al., 2006) and Amsterdam Water Dune population in the Netherlands (Blacquière et al., 2019). In most of these honey bee populations, the bees' resilience against the ectoparasite is mainly explained by the heritable trait known as Suppressed Mite Reproduction (SMR), which significantly hinders the reproductive successes of *Varroa* mites within these colonies (Blacquière et al., 2019; Locke et al., 2012; Oddie et al., 2017).

In 2019, eight single nucleotide polymorphisms (SNPs) in the honey bee exome were found to be associated with *Varroa* non-reproduction in drone brood, also referred to as Drone Brood Resistance (DBR) (Broeckx et al., 2019). The eight-variant model from this study successfully predicted 88 % of the drone phenotypes (reproduction or non-reproduction of included mite), based on their genotypes only. However, this study was limited to the drones from a single hybrid *A. m. mellifera* −derived queen from Amsterdam Dune population in the Netherlands. A subsequent population-wide screening on Flemish honey bee colonies (mainly *Apis mellifera carnica*) validated the relevance of the eight genetic variants (SNPs), enabling the construction of an adjusted eight variant model that successfully predicted 69.4 % of the drone phenotypes correctly (Lefebre et al., 2024a). Interestingly, this latter study could reduce the previously identified eight genetic variants to a three-variant model, enabling the successful prediction of up to 76 % of the drone phenotypes, which facilitates large-scale colony screening. Nevertheless, the study of Lefebre et al. (2024a) revealed variations in the predictive properties of certain variants. Some variants that were identified as protective indicators in the single Dutch *A. m. mellifera* −derived colony became risk indicators in the Flemish screening considering mainly *A. m. carnica*, and vice versa. In a follow-up study (Lefebre et al., 2024b), the allele frequencies of the eight genetic markers were assessed in over 360 *A. mellifera* colonies throughout Europe, and the results suggested that the variant type allele frequencies are mainly correlated with the subspecies of *A. mellifera* or its phylogenetic lineage. These findings indicate that the predictive properties of the genetic variants may differ across bee populations, underscoring the need to study their occurrence and variability in genetically and environmentally diverse honey bee subspecies.

The presence of *V. destructor* has also been confirmed in many African countries (Pirk et al., 2016). Unlike European subspecies, honeybees in African countries such as Uganda, Kenya and Ethiopia suffer less from *Varroa* mite infestations (Begna et al., 2016; Chemurot, 2017; Muli et al., 2014; Strauss et al., 2016). Although the resilience of African bees to *Varroa* is mainly attributed to the SMR trait, which has been observed in several bee populations from Ethiopia (Gebremedhn et al., 2019), Kenya (Nganso et al., 2018), and South Africa (Strauss et al., 2016), little is known about how the eight genetic variants identified by Broeckx et al., (2019) are distributed in honeybees across the much diversified African ecoregions with their specific genetic backgrounds (Aglagane et al., 2023; Franck et al., 2001; Themudo et al., 2020). This study determined allele frequencies of the eight genetic variants (SNPs) associated with DBR in various *Apis mellifera* subspecies populations of selected Eastern and Western African countries. In this way, this study discloses the potential of elucidating the predictive properties and potentials of these genetic variants in African honeybee subspecies by examining genotype-phenotype associations. In this way, this study discloses the potential of elucidating the predictive properties and prospects of these genetic variants in African honeybee subspecies. By examining genotype-phenotype associations in future studies, this work could facilitate large-scale screening for Varroa-resistant colonies in Africa.

2. Materials and methods

2.1. Sample collection and transportation

Adult worker bee samples were collected from different colonies in Ethiopia (ET, N = 20), Uganda (UG, N = 30) and Benin (BJ, N = 30), located in East-, East-, and West Africa, respectively. In Ethiopia, samples were collected during the active season between August and October 2017, from 10 apiaries (two colonies each) across three different agroecological zones: highland, lowland, and mid-highlands (Haftom et al., 2019). For Uganda, samples of adult bees were collected from two agroecological zones: the eastern and western highlands (Chemurot, 2017). Samples from Benin were collected across the entire country (Amakpe, 2016). After collection, the sample vials, with (BJ, UG) or without (ET) ethanol, were shipped to the Laboratory of Molecular Entomology and Bee Pathology (ʟ-MEB) (UGent, Belgium) for analysis, either at room temperature (BJ, UG) or on dry ice (ET). Once the samples arrived at ʟ-MEB, they were stored at −20 °C until further analysis. Detailed information on sample collection, transport, and storage of samples from Ethiopia, Uganda and Benin can be found in Gebremedhn et al., (2020), Chemurot, (2017) and Amakpe, (2016), respectively.

2.2. Sample preparation and DNA extraction

For each sampled colony, 30 hind legs from 30 different worker bees were dissected from the thorax using sterile tweezers and pooled in an Eppendorf tube with screw cap. For the samples collected from Benin and Uganda, the ethanol was removed, and the hind legs were rehydrated by washing them three times overnight with 800 μL 1 × PBS. After removing the last volume of PBS, 180 μL ATL buffer (QIAamp® DNA Micro Kit (Qiagen)) was added and the samples were incubated overnight at 56 °C with 20 μL proteinase K (20 mg/mL). Next, genomic DNA (gDNA) was extracted from the pooled legs following the manufacturer's instructions. Finally, the DNA was eluted in 50 μL DNase/RNase free water.

2.3. Determination of allele frequencies using qPCR with dual-labeled probes

For each gDNA sample, eight qPCR assays with dual-labeled probes (Boúúaert et al., 2021) were performed in a total reaction volume of 10 μL, containing 1 × KEY buffer, 250 nM of each primer, 250 nM of each dual-labeled probe, 200 μM of each dNTP, 0.5 U TEMPase Hot Start DNA Polymerase (VWR) and 20 ng gDNA (Boúúaert et al., 2021). Per genotyping assay, a SNP-specific calibration curve with standards of 0 %, 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % and 100 % Vt allele

(variant type allele) was run in duplicate (constructed similarly as in Lefebre et al. (2024b). The qPCR assays were run on the Bio-Rad C1000TM Thermal Cycler with CFX96TM Real-Time System with the following cycling settings: 95 °C for 14′40″, 60 × (95 °C for 20″ and 40″ of the assay-specific annealing temperature) (Boúúaert et al., 2021).

Data analysis was performed with the Bio-Rad CFX Manager 3.1 Software and Microsoft Excel. For each pooled worker bee sample, the percentage of variant allele (%Vt) was calculated using retrograde regression analysis of the ratio [end RFU FAM/end RFU TR] of the sample compared to those in the biquadratic intraplate calibration curve. The results were further refined by applying the ratio [end RFU TR/end RFU FAM], consistent with the method used by Lefebre et al. (2024b).

2.4. Data analysis

Data representation and statistical analysis was performed in R (version 4.4.1) and RStudio (version 2023.12.1 + 402) with the ggplot2 package (v 3.5.1). Differences in allele frequencies of the variants among samples from Benin, Ethiopia and Uganda were determined using the non-parametric Kruskal-Wallis test. If significant, post-hoc Dunn's tests with Bonferroni-correction were applied. Principal Component Analysis (PCA) was performed using the FactoMineR (v 2.11) and factoextra (v 1.0.7) packages in R. Cos2 and contribution values were used to determine how the variables (i.e. SNPs) contributed to the principal components (Fig. S1). The k-means method was used for cluster analysis. The optimal number of clusters was determined using the Within Sum of Square (WSS)-, silhouette-, and gap statistic methods (Fig. S2).

3. Results

3.1. Allele frequency distributions

The screening revealed high variant type allele frequencies for SNP2 (avg. 75–84 %) and SNP8 (avg. 99–100 %) across all African honeybee samples (Fig. 1, Table S1). In contrast, SNP1 (avg. 0–1.2 %), SNP6 (avg. 0–6.5 %), and SNP7 (avg. 0 %) showed very low variant type allele frequencies in the African honeybee samples. Moreover, significant differences in variant type allele frequencies for SNP1, SNP3, SNP5 and SNP6 were observed between the countries of origin of the African samples (Benin (BJ), Ethiopia (ET) and Uganda (UG)) (Fig. 1, Table S2). The most notable difference was found for SNP5, which showed very

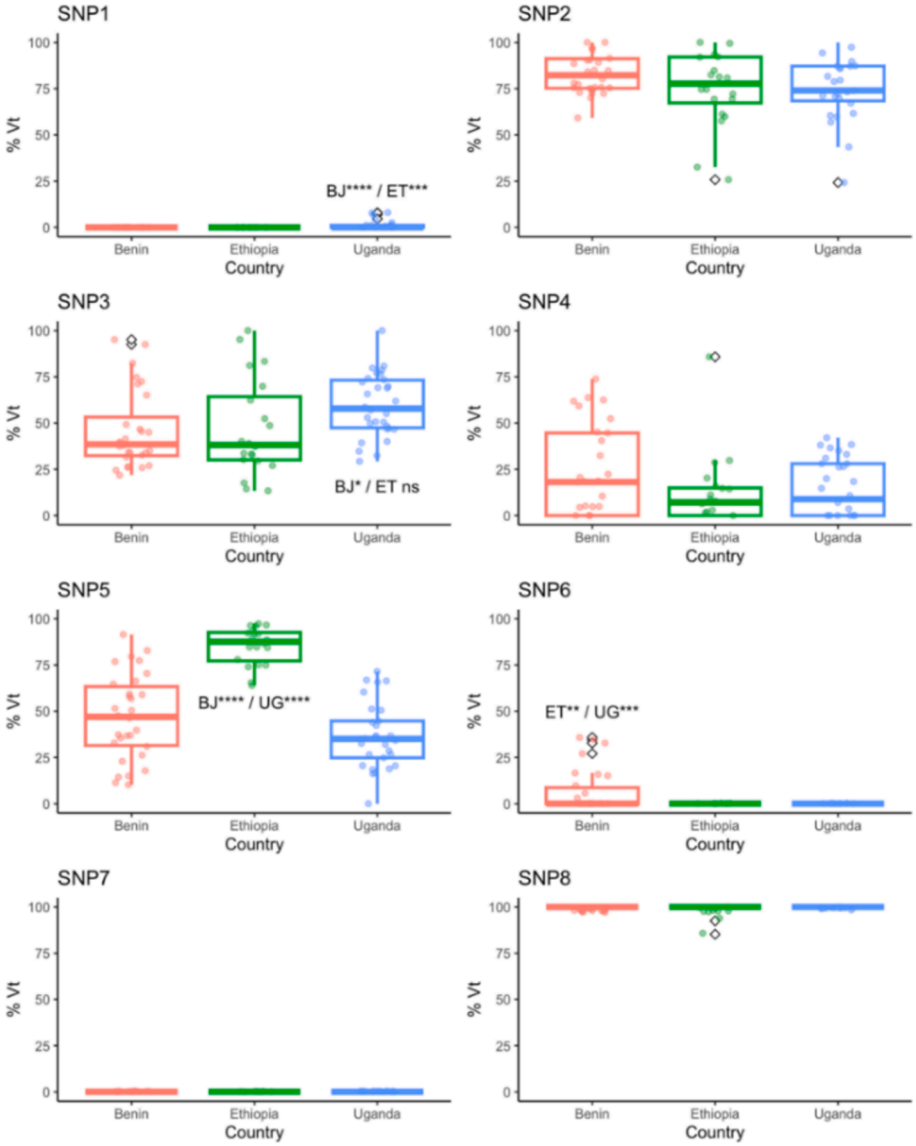

Fig. 1. Variant type allele frequencies (%Vt) for the eight DBR-associated genetic variants (SNPs) in pooled worker bee samples from Benin (BJ; N = 30), Ethiopian (ET; N = 20) and Ugandan (UG; N = 30) colonies. Bonferroni-corrected significance levels: ns = not significant; p ≤ 0.05*; p ≤ 0.01**; p ≤ 0.001***; p ≤ 0.0001****.

high variant type allele frequencies in Ethiopian bees (ET; avg. 85.3 %) compared to those from Benin (BJ; avg. 46.9 %) and Uganda (UG; avg. 36.6 %)) ($P < 0.0001$).

3.2. Allele frequency patterns in African honeybees compared to European lineages

To assess similarities in allele frequency distributions between the investigated African honeybee samples and European honeybee lineages (A, C, and M) screened by Lefebre et al. (2024b), we performed Principal Component Analysis (PCA) and clustering analysis using data from Lefebre et al. (2024b). Their study revealed strong correlations between variant type allele frequencies (%Vt) for certain SNPs and the samples' subspecies or genetic lineage.

To extend the current analysis, the allele frequency data set from genetic lineages sampled in the European study of Lefebre et al. (2024b) were incorporated: A line (*A. mellifera iberiensis*; N = 7), M line (*A. m. mellifera*; N = 37) and C line (*A. mellifera adami*; N = 2, *A. mellifera carnica*; N = 62, *A. mellifera carpatica*; N = 4 *and A. mellifera ligustica;* N = 27). This broader dataset enabled us to evaluate how the variant type allele frequencies of the eight genetic markers correlate with the different genetic lineages in Europe, helping to identify the related lineages for the sampled African populations. Thus, in the current study, PCA was performed on this entire data set of 219 pooled worker bee samples (139 from Europe, 80 from Africa).

The cos2 – and contribution values of the PCA indicated that the first two principal components or dimensions (Dim 1 and Dim 2) capture a substantial portion of the total variation observed in the dataset, as both explain nearly 55.2 % (39.2 % and 16 %, respectively) of the total variance (Fig. S1, Fig. 2). In the first dimension (Dim 1), SNP1, SNP4, SNP6, and SNP8 were the most significant contributing variants, while SNP2 and SNP5 contributed most to the second dimension (Dim 2) (Fig. S1; arrows on Fig. 2). Since almost no differences in percentages of variant type allele for SNP3 and SNP7 were observed between subspecies in the European screening (Lefebre et al., 2024b), or in the African sample groups (Fig. 1), we opted to consider only the first two dimensions for further analyses (Fig. 2).

The two-dimensional PCA showed high grouping of the African honeybee samples in the PCA biplot (Fig. 2). In the first dimension (Dim 1), representing SNP1, SNP4, SNP6 and SNP8, all African honeybee samples showed coordinates similar to the samples from the A lineage

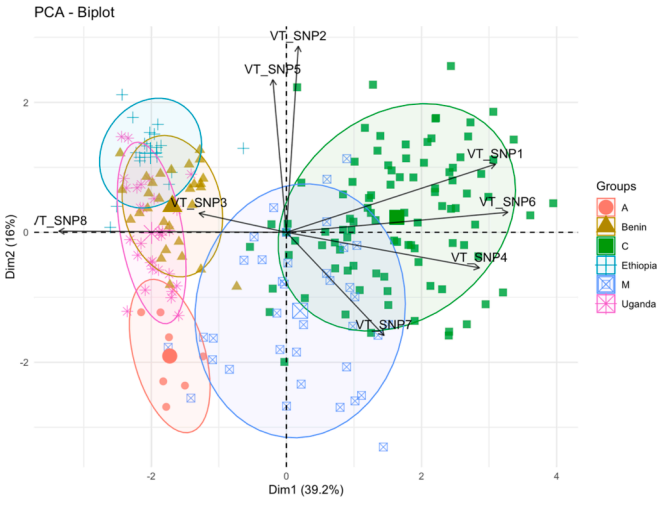

Fig. 2. PCA of variant type allele frequencies of the eight genetic variants associated with DBR, for the African samples from Benin, Ethiopia and Uganda, extended with data from European lineages (from (Lefebre et al., 2024b)). This PCA focuses only on the first two principal components (Dim1 and Dim2). Ellipses are drawn at 80% confidence interval.

(*A. mellifera iberiensis*) and some samples from the M lineage (*A. m. mellifera*). In contrast, the samples from Benin, Ethiopia and Uganda differ the most from those of the C lineage in this first dimension. Based on these findings, it is possible that the African samples share high genetic similarities with the European A lineage (*A. mellifera iberiensis*).

In the second dimension (Dim2), which represents SNP2 and SNP5, the honeybee samples from Ethiopia show slightly higher coordinates than those from Benin and Uganda. This difference may be attributed to the significantly higher percentages of variant allele for SNP5 in the Ethiopian samples. When compared to European samples of the A lineage (ssp. *iberiensis*), the honeybee African samples showed higher coordinates in the second dimension (Fig. 2). This difference may be attributed to the very low percentages of variant type allele for SNP2 in samples of the ssp. *iberiensis*, which contrasts with the high percentages of variant type alleles for this SNP in the African honeybee samples (*cf.* Fig. 1).

The optimal number of clusters for grouping the screened samples based on PCA coordinates was determined using the Within Sum of Square (WSS), Silhouette and Gap statistic method (Fig. S2). All three methods indicated that the optimal number of clusters is two (k = 2) (Fig. 3). However, to assess whether the clusters or grouping of the honeybee samples remained consistent across different values of k, we performed the clustering analysis using k = 2, k = 3 and k = 4 (Fig. 3).

When k-means clustering with k = 2 was applied, all African samples were grouped together with all samples from the A lineage (ssp. *iberiensis*). This may suggest a possible genetic connection between both lines. Additionally, some samples from the M lineage (ssp. *mellifera*) and a few samples from the C lineage were also included in this cluster (Fig. 3; k = 2). Clustering with k = 3 results in three distinct groups: one cluster containing mainly C lineage samples, another cluster predominantly containing samples belonging to the M and A lineages, and a third cluster mainly containing the samples from Africa (Fig. 3). Notably, the African samples continue to group with some samples from the A lineage.

Increasing the number of clusters to 4 (k = 4) renders a cluster mainly containing samples from the C lineage, a cluster containing samples from lineages C and M, one cluster containing samples from the A lineage and some samples from Benin and Uganda, and one cluster with all remaining African honeybee samples (Fig. 3). However, when the analysis expanded to k = 4, all Ethiopian bees clustered separately from the A, C, and M lineages.

4. Discussion

4.1. Allele frequency distributions

Our results reveal that three variants, particularly SNP2 (75–84 %), SNP5 (36.6 %-85.3 %) and SNP8 (99–100 %), identified as risk variants in the hybrid *A. m. mellifera* –derived colony by Broeckx et al., (2019), are highly prevalent in the honey bee populations from Ethiopia, Uganda and Benin. On the other hand, the present study revealed low allele frequencies for SNP6 and SNP7 in the African bee populations, which were protective indicators in the single *A. m. mellifera* –derived colony (Broeckx et al., 2019). Risk variants in *A. m. mellifera* (M lineage) and *A. m. carnica* (C lineage) are associated with a higher likelihood of successful *Varroa* mite reproduction in drone brood, which in turn promotes the growth of the *Varroa* mite population (Broeckx et al., 2019; Lefebre, Broeckx, et al., 2024). Considering the resilience of African bees to *Varroa* mite infestations (Begna et al., 2016; Chemurot, 2017; Muli et al., 2014; Strauss et al., 2016), the SNPs with high and low variant allele frequencies in African bees may serve as protective and risk indicators, respectively, for these subspecies. Shifts in the properties of these variants—whether they act as risk indicators or protective indicators—may be dependent of the subspecies (Lefebre et al., 2024a). In line with this hypothesis, two genetic variants previously identified as risk indicators by Broeckx et al. (2019) in the *A. m. mellifera* –derived

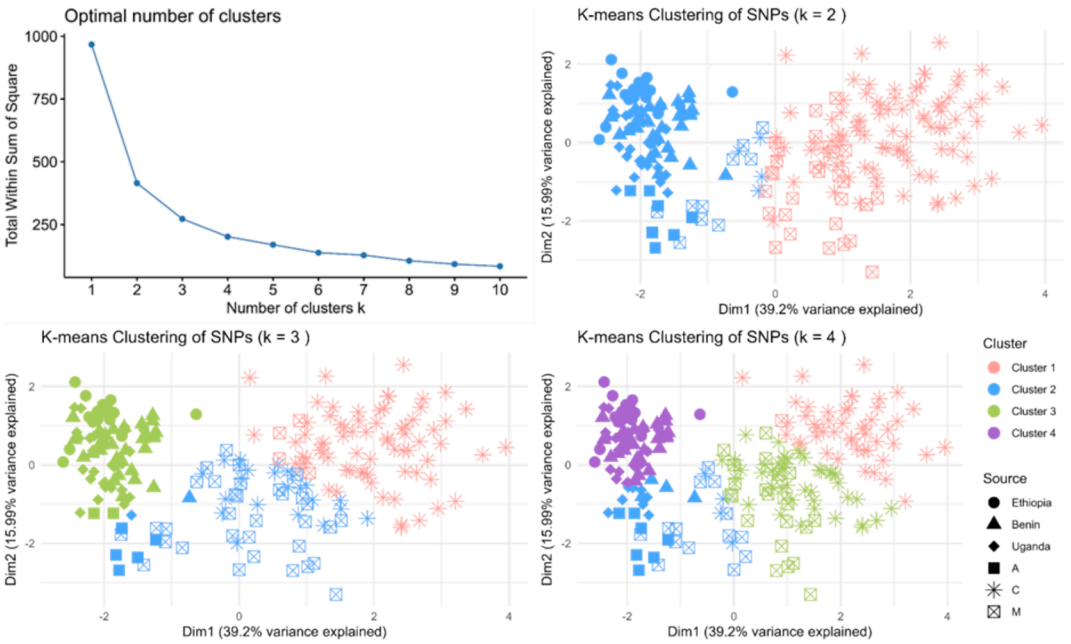

Fig. 3. k-means clustering of African and European honeybee samples in the first two PCA dimensions (Dim1 and Dim2). The optimal number of clusters k = 2 (see Fig. S2 for all three methods). However, clustering was performed with k = 2, k = 3 and k = 4 to assess whether the clusters remained consistent across different values of k.

colony, annotated as SNP2 and SNP8 (Boúúaert et al., 2021), were found to have shifted to protective indicators in Flemish *A. m. carnica* of the C lineage. Conversely, a variant described as a protective indicator in the original study (Broeckx et al. 2019), annotated as SNP6 (Boúúaert et al., 2021), also shifted to being a risk indicator in Flemish *A. m. carnica* (Lefebre et al., 2024a). These results suggest that the roles of these variants differ across honeybee populations and vary across subspecies or even genetic lineages, highlighting the need for phenotype-genotype association studies.

This study also showed significant differences in variant type allele frequencies of the different variants among African bee samples of different origin (Table S2). This may indicate genetic variation within these populations (Andrews, 2010), which aligns with the previous studies that indicate the presence of high genetic diversity of bees in Africa (Aglagane et al., 2023; Franck et al., 2001; Themudo et al., 2020). Genetic variation is a crucial factor for colony survival under various environmental stressors (Leclercq et al., 2018; Themudo et al., 2020) and may enhance the resilience of African bees against the *Varroa* mite and associated pathogens. In the European study, variability in variant type allele frequencies of the different variants has also been reported among different honey bee subspecies and within the same subspecies, but sampled across different geographical locations (Lefebre et al., 2024b). Based on these findings, the variability in variant type allele frequencies of the different variants in African bees may be attributed to the difference in environmental conditions and their interaction with the bees' genetic background. In line with this, the samples from Ethiopia showed slightly higher coordinates in the PCA for Dim 2 compared to those from Benin and Uganda, likely due to significantly higher percentages of variant alleles for SNP5 found in Ethiopian bees. These findings confirm that Ethiopian bees may possess distinct genetic traits that differentiate them from other African honeybee populations.

4.2. Allele frequency patterns in African honeybees compared to European lineages

According to Lefebre et al. (2024b), the allele frequencies of the eight genetic variants associated with DBR are closely linked to the subspecies or lineage of A. mellifera. Therefore, the present study analyzed the relatedness of African honey bee samples to European lineages (A, C, and M lineage) (Lefebre et al., 2024b; Themudo et al., 2020; Tihelka et al., 2020) by incorporating allele frequency data of the variants from European populations and subsequent exploration by PCA.

According to Dim 1, which explains most of the variance (39.2 %) and represents SNP1, SNP4, SNP6 and SNP8, most of the African samples grouped closely to those from the *ssp. Iberiensis* lineage A. In line with this, the variant type allele frequencies of six variants (SNP1, SNP3, SNP4, SNP6, SNP7, and SNP8) are similar between the African honeybee samples and those reported for ssp. *Iberiensis* from Portugal (Lefebre et al., 2024b). These results probably demonstrated the genetic similarities between African honeybees and *ssp. Iberiensis* (Andrews, 2010), the latter being known for its high resistance to *Varroa* mites in Portugal, primarily attributed to the SMR trait (Mondet et al., 2020). A key difference between the two populations is that the variant type allele frequency for SNP2 is significantly high (75–84 %) in African honeybees, while it is completely absent in *ssp. Iberiensis* (0 %) (Lefebre et al., 2024b). In line with this, in the second dimension, which primarily represents SNP2 and SNP5, the African samples exhibited higher coordinates than samples from *ssp. Iberiensis*, indicating notable genetic differences (Andrews, 2010). Therefore, while these two groups shared some genetic traits, African honeybees may possess unique protective alleles that enhance their resistance to *Varroa* mites.

The O, M, and C lineages are destributed throughout the Middle East, Northern and Western Europe, and Southeastern Europe, respectively (Aglagane et al., 2023; Franck et al., 2001; Themudo et al., 2020; Tihelka et al., 2020). The honey bee A lineage is prevalent throughout nearly all African regions, except Ethiopia (Aglagane et al., 2023; Franck et al., 2001; Themudo et al., 2020; Tihelka et al., 2020), as well as in Portugal and southwestern Spain (Tihelka et al., 2020).

At k = 2, all African samples formed a distinct cluster together with all samples from the European A lineage (Lefebre et al., 2024b). At k = 3, the African samples formed clusters alongside some samples from the A lineage and a few from the M lineage. However, at k = 4, some samples from Benin and Uganda continued to align closely with the A lineage, while a few were associated with the M lineage. Similarly, the phylogenetic analyses conducted by Amakpe et al., (2018) clustered honey bee samples from Benin within the evolutionary A lineage. According to

Kasangaki et al., (2017), there are two honey bee races, *A. m. adansonii* and *A. m. cutellate,* in Uganda, both of which belong to the A lineage of honeybees (Frazier et al., 2024). However, at k = 4, the genetic relatedness of the African honeybee samples did not remain consistent. Notably, at k = 4, none of the Ethiopian samples showed relatedness to the known lineages of honeybees (A, C, and M), while some samples from Benin and Uganda did cluster together with mainly samples from the A-lineage. In line with this, the samples from Ethiopia showed higher coordinates in the second dimension (Dim2) than those from Benin and Uganda. These results may indicate that the Ethiopian bees are distinct at the lineage level, and that they differ from populations in neighboring geographic regions. These findings strengthen the claim that Ethiopian honeybees belong to a unique lineage known as the Y lineage (Aglagane et al., 2023; Franck et al., 2001; Themudo et al., 2020). Recent studies on the classification and distribution of honey bee subspecies in Ethiopia using morphometric and genetic analyses also proposed a unique subspecies called *A. m. simensis* (Hailu et al., 2020; Meixner et al., 2011), belonging to the unique Y lineage (Tihelka et al., 2020).

In this study, we examined the allelic frequencies and distribution of eight SNPs in African *A. mellifera* subspecies and performed clustering analysis based on these frequencies and the data from Lefebre et al., (2024a). The observed clustering patterns most probably originate from the genetic relatedness between the honey bee subspecies, as genetically related subspecies typically exhibit similar allele frequency distributions for genetic variants (Lefebre et al., 2024b). In other words, while our analysis is based on a limited number of SNPs, the obtained results already reflect the potential genetic relationships between the analyzed samples. Future studies with a more comprehensive set of genetic markers would be valuable for a more robust analysis of genetic relatedness among these subspecies.

5. Conclusions

This study provides deep insights into the allele frequencies of eight genetic variants (SNPs) associated with DBR in European bees within Eastern and Western African honey bee populations. It reveals the presence of seven out of eight variants in African *A. mellifera* subspecies, suggesting these variants may contribute to their natural resistance to *Varroa* mite infestations. African honeybees were found to cluster together with samples from the A lineage, specifically the subspecies *A. m. iberiensis*. Overall, the presence of most DBR-associated genetic variants in African honeybee populations opens opportunities to elucidate the predictive properties and potential of these genetic variants in honey bee subspecies on the African continent.

Although this study revealed the occurrence and allele frequencies of genetic variants (SNPs) associated with SMR in African bees, phenotype-genotype associations were not studied. Hence, further research should investigate the predictive power of the selected SNPs for *Varroa*-resistant honeybee lines through phenotype-genotype analysis. This additional work would provide a more comprehensive understanding of the relationship between these genetic markers and the SMR trait in African honeybee populations.

CRediT authorship contribution statement

Haftom Gebremedhn: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Regis Lefebre:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Moses Chemurot:** Writing – review & editing, Data curation. **Felicien Amakpe:** Writing – review & editing, Data curation. **Bezabeh Amssalu:** Writing – review & editing. **Lina De Smet:** Writing – review & editing, Conceptualization. **Dirk C. de Graaf:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors express their appreciation to Ghent University—Special Research Fund (BOF) (Award Number: 001W14316 | Recipient: Gebremedhn Haftom), Global Minds | Recipient: Gebremedhn Haftom), and the Tigray Agricultural Research Institute | Recipient: Gebremedhn Haftom) for their financial support, as well as to the beekeepers, technicians, experts, and researchers in the Tigray National Regional State for their cooperation and assistance.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2025.108276.

References

Aglagane, A., Oleksa, A., Er-Rguibi, O., Tofilski, A., El Mouden, E.H., Aamiri, A., Aourir, M., 2023. Genetic diversity and population structure of the Saharan honey bee *Apis mellifera* sahariensis from southeastern Morocco: introgression assessment and implications for conservation. Apidologie 54 (31). https://doi.org/10.1007/s13592-023-01009-9.

Al-Ghamdi, A.A., Adgaba, N., Herab, A.H., Ansari, M.J., 2017. Comparative analysis of profitability of honey production using traditional and box hives. Saudi J. Bio. Sci. 24 (5), 1075–1080. https://doi.org/10.1016/j.sjbs.2017.01.007.

Amakpe, F., De Smet, L., Brunain, M., Jacobs, F.J., Sinsin, B., de Graaf, D.C., 2018. Characterization of native honey bee subspecies in Republic of Benin using morphometric and genetic tools. J. Apicultural Sci. 62 (1). https://doi.org/10.2478/JAS-2018-0006.

Amakpe, F. (2016). Exploring the beekeeping potential in the Republic of Benin by examining the melliferous trees, the honeybees and the honey they produce [Ghent University]. https://biblio.ugent.be/publication/8520008/file/8520011.

Andrews, C.A., 2010. Natural selection, genetic drift, and gene flow do not act in isolation in natural populations. Nat. Educ. Knowledge 3 (10), 5.

Barroso-Arévalo, S., Fernández-Carrión, E., Goyache, J., Molero, F., Puerta, F., Sánchez-Vizcaíno, J.M., 2019. High load of deformed wing virus and *Varroa destructor* Infestation are related to weakness of honey bee colonies in southern Spain. Front. Microbiol. 10 (1331). https://doi.org/10.3389/fmicb.2019.01331.

Begna, D., Gela, A., Negera, T., Bezabeh, A., 2016. Identifying the species, effects and seasonal dynamics of honeybee varroa mites: a newly emerging parasite to Ethiopian honeybee. Int. J. Scientif. Res. Environ. Sci. Toxicol. 1 (1), 4.

Benaets, K., Geystelen, A.V., Cardoen, D., Smet, L.D., De Graaf, D.C., Schoofs, L., Larmuseau, M.H.D., Brettell, L.E., Martin, S.J., Wenseleers, T., 2017. Covert deformed wing virus infections have long-term deleterious effects on honeybee foraging and survival. Proc .R. Soc.b 284 (20162149). https://doi.org/10.1098/rspb.2016.2149.

Blacquière, T., Boot, W., Calis, J., Moro, A., Neumann, P., Panziera, D., 2019. Darwinian black box selection for resistance to settled invasive *Varroa destructor* parasites in honey bees. Biol. Invasions 21, 2519–2528. https://doi.org/10.1007/s10530-019-02001-0.

Boúúaert, D.C., Van Poucke, M., De Smet, L., Verbeke, W., de Graaf, D.C., Peelman, L., 2021. qPCR assays with dual-labeled probes for genotyping honey bee variants associated with varroa resistance. BMC Vet. Res. 17 (179). https://doi.org/10.1186/s12917-021-02886-x.

Bowen-Walker, P.L., Martin, S., Gunn, A., 1999. The transmission of deformed wing virus between honeybees (*Apis mellifera*L.) by the Ectoparasitic MiteVarroa jacobsoniOud. J. Invertebr. Pathol. 73 (1), 101–106. https://doi.org/10.1006/jipa.1998.4807.

Bradbear, N. (2009). Bees and their role in forest livelihoods: a guide to the services provided by bees and the sustainable harvesting, processing and marketing of their products. In *Non-wood Forest Products 19*. FAO, Rome, Italy.

Brodschneider, R., Schlagbauer, J., Arakelyan, I., Ballis, A., Brus, J., Brusbardis, V., Cadahía, L., Charrière, J.D., Chlebo, R., Coffey, M.F., Cornelissen, B., da Costa, C.A., Danneels, E., Danihlík, J., Dobrescu, C., Evans, G., Fedoriak, M., Forsythe, I., Gregorc, A., Gray, A., 2023. Spatial clusters of *Varroa destructor* control strategies in Europe. J. Pest. Sci. 96 (2), 759–783. https://doi.org/10.1007/s10340-022-01523-2.

Broeckx, B.J.G., De Smet, L., Blacquière, T., Maebe, K., Khalenkow, M., Van Poucke, M., Dahle, B., Neumann, P., Bach Nguyen, K., Smagghe, G., Deforce, D., Van Nieuwerburgh, F., Peelman, L., de Graaf, D.C., 2019. Honey bee predisposition of resistance to ubiquitous mite infestations. Sci. Rep. 9 (7794). https://doi.org/10.1038/s41598-019-44254-8.

Brutscher, L.M., Mcmenamin, A.J., Flenniken, M.L., 2016. The buzz about honey bee viruses. Plant Pathol. 12 (8). https://doi.org/10.1371/journal.ppat.1005757.

Büchler, R., Berg, S., Le Conte, Y., 2010. Breeding for resistance to *Varroa destructor* in Europe. Apidologie 41 (3), 393–408. https://doi.org/10.1051/apido/2010011.

Calderone, N.W., 2012. Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992–2009. PLoS One 7 (5). https://doi.org/10.1371/journal.pone.0037235.

Chemurot, M. (2017). The distribution , infestation levels and effects of honeybee parasites and pathogens on colony performance in two agro-ecological zones of Uganda [Ghent University]. https://biblio.ugent.be/publication/8519019/file/8519026.pdf.

Currie, R.W., Pernal, S.F., Guzmán-Novoa, E., 2010. Honey bee colony losses in Canada. J. Apic. Res. 49 (1), 104–106. https://doi.org/10.3896/IBRA.1.49.1.18.

DeGrandi-Hoffman, G., Chen, Y., 2015. Nutrition, immunity and viral infections in honey bees. Curr. Opin. Insect Sci. 10, 170–176. https://doi.org/10.1016/j.cois.2015.05.007.

Espregueira Themudo, G., Rey-Iglesia, A., Robles Tascón, L., Bruun Jensen, A., da Fonseca, R.R., Campos, P.F., 2020. Declining genetic diversity of European honeybees along the twentieth century. Sci. Rep. 10 (10520). https://doi.org/10.1038/s41598-020-67370-2.

Franck, P., Garnery, L., Loiseau, A., Oldroyd, B.P., Hepburn, H.R., Solignac, M., Cornuet, J., 2001. Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data. Heredity 86, 420–430.

Frazier, M., Muli, E., Patch, H., 2024. Ecology and management of African honey bees (*Apis mellifera* L.). Annu. Rev. Entomol. 69, 439–453. https://doi.org/10.1146/annurev-ento-020823-095359.

Fries, I., Imdorf, A., Rosenkranz, P., 2006. Survival of mite infested (*Varroa destructor*) honey bee (*Apis mellifera*) colonies in a Nordic climate. Apidologie 37 (5), 564–570. https://doi.org/10.1051/apido:2006031.

Garratt, M.P.D., Breeze, T.D., Jenner, N., Polce, C., Biesmeijer, J.C., Potts, S.G., 2014. Avoiding a bad apple: Insect pollination enhances fruit quality and economic value. Agr Ecosyst Environ 184, 34–40. https://doi.org/10.1016/j.agee.2013.10.032.

Gebremedhn, H., Amssalu, B., De De Smet, L., de Graaf, D.C., 2019. Factors restraining the population growth of *Varroa destructor* in Ethiopian honey bees (*Apis mellifera* simensis). PLoS One 14 (9). https://doi.org/10.1371/journal.pone.0223236.

Gebremedhn, H., Deboutte, W., Schoonvaere, K., Demaeght, P., De Smet, L., Amssalu, B., Matthijnssens, J., de Graaf, D., 2020. Metagenomic approach with the NetoVIR enrichment protocol reveals virus diversity within Ethiopian honey bees (*Apis mellifera* simensis). Viruses 12 (1218). https://doi.org/10.3390/v12111218.

A. Gray R. Brodschneider N. Adjlane A. Ballis V. Brusbardis J. Charrière R. Chlebo F. Coffey M., Cornelissen, B., Amaro da Costa, C., Csáki, T., Dahle, B., Danihlík, J., Dražić, M. M., Evans, G., Fedoriak, M., Forsythe, I., de Graaf, D., Gregorc, A., Soroker, V. Loss rates of honey bee colonies during winter 2017/18 in 36 countries participating in the COLOSS survey, including effects of forage sources J. Apic. Res. 58 4 2019 479 485 10.1080/00218839.2019.1615661.

Haftom, H., Haftu, A., Goitom, K., Meseret, H., 2019. Agroclimatic zonation of Tigray region of Ethiopia based on aridity index and traditional agro-climatic zones. J. Agrometeorol. 21 (2), 176–181.

Hailu, T.G., D'Alvise, P., Tofilski, A., Fuchs, S., Greiling, J., Rosenkranz, P., Hasselmann, M., 2020. Insights into Ethiopian honey bee diversity based on wing geomorphometric and mitochondrial DNA analyses. Apidologie 51 (6), 1182–1198. https://doi.org/10.1007/s13592-020-00796-9.

Kasangaki, P., Nyamasyo, G., Ndegwa, P., Kajobe, R., Angiro, C., Kato, A., Masembe, C., 2017. Mitochondrial DNA (mtDNA) markers reveal low genetic variation and the presence of two honey bee races in Uganda's agro-ecological zones. J. Apic. Res. 56 (2), 112–121. https://doi.org/10.1080/00218839.2017.1287997.

Kevill, J.L., De Souza, F.S., Sharples, C., Oliver, R., Schroeder, D.C., Martin, S.J., 2019. DWV-A Lethal to Honey Bees (*Apis mellifera*): A Colony Level Survey of DWV Variants (A, B, and C) in England, Wales, and 32 States across the US. Viruses 11 (426). https://doi.org/10.3390/v11050426.

Klein, A., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B Biol. Sci. 274 (1608), 303–313. https://doi.org/10.1098/rspb.2006.3721.

Le Conte, Y., De Vaublanc, G., Crauser, D., Jeanne, F., Rousselle, J.C., Bécard, J.M., 2007. Honey bee colonies that have survived *Varroa destructor*. Apidologie 38, 566–572. https://doi.org/10.1051/apido:2007040.

Leclercq, G., Gengler, N., Francis, F., 2018. How human reshaped diversity in honey bees (*Apis mellifera* L.): a review. Entomologie Faunistique-Faunistic Entomology 71.

Lefebre, R., Broeckx, B.J.G., De Smet, L., Peelman, L., de Graaf, D.C., 2024a. Population-wide modelling reveals prospects of marker-assisted selection for parasitic mite resistance in honey bees. Sci. Rep. 14 (7866). https://doi.org/10.1038/s41598-024-58596-5.

Lefebre, R., De Smet, D., Tehel, A., Paxton, R.J., Bossuyt, E., Verbeke, W., van Dooremalen, C., Ulgezen, Z.N., van den Bosch, T., Schaafsma, F., Valkenburg, D., Olio, R.D., Alaux, C., Dezmirean, D.S., Giurgiu, A.I., Capela, N., Sim, S., Sousa, P., Bencsik, M., de Graaf, D.C., 2024b. Allele frequencies of genetic variants associated with Varroa drone brood resistance (DBR) in *Apis mellifera* subspecies across the European continent. Insects 15 (419).

Locke, B., Le Conte, Y., Crauser, D., Fries, I., 2012. Host adaptations reduce the reproductive success of *Varroa destructor* in two distinct European honey bee populations. Ecol. Evol. 2 (6), 1144–1150. https://doi.org/10.1002/ece3.248.

Martin, S.J., Highfield, A.C., Brettell, L., Villalobos, E.M., Budge, G.E., Powell, M., Nikaido, S., Schroeder, D.C., 2012. Global honey bee viral landscape altered by a parasitic mite. Science 336 (6086), 1304–1306. https://doi.org/10.1126/science.1220941.

Meixner, M.D., Leta, M.A., Koeniger, N., Fuchs, S., 2011. The honey bees of Ethiopia represent a new subspecies of *Apis mellifera*—*Apis mellifera* simensis n. ssp. Apidologie 42 (3), 425–437. https://doi.org/10.1007/s13592-011-0007-y.

Mondet, F., Parejo, M., Meixner, M.D., Costa, C., Kryger, P., Bigio, G., Eliza, C., Cebotari, V., Dahle, B., 2020. Evaluation of Suppressed Mite Reproduction (SMR) reveals potential for Varroa resistance in European honey bees (*Apis mellifera* L.). Insects 11 (595). https://doi.org/10.3390/insects11090595.

Muli, E., Patch, H., Frazier, M., Frazier, J., Torto, B., Baumgarten, T., Kilonzo, J., Kimani, J.N., Mumoki, F., Masiga, D., Tumlinson, J., Grozinger, C., 2014. Evaluation of the distribution and impacts of parasites, pathogens, and pesticides on honey bee (*Apis mellifera*) populations in East Africa. PLoS One 9 (4). https://doi.org/10.1371/journal.pone.0094459.

Neov, B., Georgieva, A., Shumkova, R., Radoslavov, G., Hristov, P., 2019. Biotic and abiotic factors associated with colonies mortalities of managed honey bee (*Apis mellifera*). Diversity 11 (237). https://doi.org/10.3390/d11120237.

Nganso, B.T., Fombong, A.T., Yusuf, A.A., Pirk, C.W.W., Stuhl, C., Torto, B., 2018. Low fertility, fecundity and numbers of mated female offspring explain the lower reproductive success of the parasitic mite *Varroa destructor* in African honeybees. Parasitology 145 (12), 1633–1639. https://doi.org/10.1017/S0031182018000616.

Oddie, M.A.Y., Dahle, B., Neumann, P., 2017. Norwegian honey bees surviving *Varroa destructor* mite infestations by means of natural selection. PeerJ 5 (e3956). https://doi.org/10.7717/peerj.3956.

Pirk, C.W.W., Strauss, U., Yusuf, A.A., Démares, F., Human, H., 2016. Honeybee health in Africa—a review. Apidologie 47 (3), 276–300. https://doi.org/10.1007/s13592-015-0406-6.

Posada-Florez, F., Childers, A.K., Heerman, M.C., Egekwu, N.I., Cook, S.C., Chen, Y., Evans, J.D., Ryabov, E.V., Honey, 2019. Deformed wing virus type A, a major honey bee pathogen, is vectored by the mite *Varroa destructor* in a non-propagative manner. Sci. Rep. 9 (12445). https://doi.org/10.1038/s41598-019-47447-3.

Ramsey, S.D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J.D., Cohen, A., Lim, D., Joklik, J., Cicero, J.M., Ellis, J.D., Hawthorne, D., VanEngelsdorp, D., 2019. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. Proc. Natl. Acad. Sci. 116 (5), 1792–1801. https://doi.org/10.1073/pnas.1818371116.

Rinderer, T.E., Harris, J.W., Hunt, G.J., De Guzman, L.I., 2010. Breeding for resistance to *Varroa destructor* in North America. Apidologie 41, 409–424. https://doi.org/10.1051/apido/2010015.

Sabbahi, R., 2022. Effects of Climate Change on Insect Pollinators and Implications for Food Security — Evidence and Recommended Actions. In: *The Food Security, Biodiversity, and Climate Nexus*. Springer International Publishing, Cham, pp. 143–163.

Sammataro, D., Untalan, P., Guerrero, F., Finley, J., 2005. The resistance of varroa mites (Acari: Varroidae) to acaricides and the presence of esterase. Int. J. Acarol. 31 (1), 67–74. https://doi.org/10.1080/01647950508684419.

Shen, M., Yang, X., Cox-Foster, D., Cui, L., 2005. The role of varroa mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. Virology 342 (1), 141–149. https://doi.org/10.1016/j.virol.2005.07.012.

Smith, K.M., Loh, E.H., Rostal, M.K., Zambrana-torrelio, C.M., Mendiola, L., Daszak, P., 2014. Pathogens, pests, and economics: drivers of honey bee colony declines and losses. Ecohealth 10, 434–445. https://doi.org/10.1007/s10393-013-0870-2.

Strauss, U., Dietemann, V., Human, H., Crewe, R.M., Pirk, C.W.W., 2016. Resistance rather than tolerance explains survival of savannah honeybees (*Apis mellifera* scutellata) to infestation by the parasitic mite *Varroa destructor*. Parasitology 143 (3), 374–387. https://doi.org/10.1017/S0031182015001754.

Tihelka, E., Cai, C., Pisani, D., Donoghue, P.C.J., 2020. Mitochondrial genomes illuminate the evolutionary history of the Western honey bee (*Apis mellifera*). Sci. Rep. 10 (14515). https://doi.org/10.1038/s41598-020-71393-0.

VanEngelsdorp, D., Hayes, J., Underwood, R.M., Pettis, J., 2008. A survey of honey bee colony losses in the U.S. PLoS One 3 (12). https://doi.org/10.1371/journal.pone.0004071. Fall 2007 to Spring 2008.

VanEngelsdorp, D., Caron, D., Hayes, J., Underwood, R., Henson, M., Rennich, K., Spleen, A., Andree, M., Snyder, R., Lee, K., Roccasecca, K., Wilson, M., Wilkes, J., Lengerich, E., Pettis, J., 2012. A national survey of managed honey bee 2010–11 winter colony losses in the USA: results from the Bee Informed Partnership. J. Apic. Res. 51 (1), 115–124. https://doi.org/10.3896/IBRA.1.51.1.14.

Yalçınkaya, A., Keskin, N., 2010. The investigation of honey bee diseases after colony losses in Hatay and Adana provinces of Turkey. Mellifera 10 (20), 24–31.