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Parasite and virus dynamics in the honeybee *Apis mellifera unicolor* on a tropical island recently invaded by *Varroa destructor*

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ABSTRACT

In La Réunion, the established honeybee subspecies *Apis mellifera unicolor,* an endemic subspecies of African lineage, is facing considerable challenges. Since the introduction of the *Varroa destructor* mite in 2017 high colony losses have been recorded. We investigated the dynamics of *V. destructor* and two viruses, the Deformed Wing Virus (DWV), known to be transmitted by the mite, and the Chronic Bee Paralysis Virus (CBPV), in *A. m. unicolor*. Colonies from two apiaries located at 300 and 900 m a.s.l were monitored twice for one year without any acaricide treatment. The brood area, *V. destructor* infestation rates, DWV and CBPV prevalence and load were recorded monthly. *A. m. unicolor* maintained brood rearing throughout the year. *Varroa destructor* infestation resulted in high colony mortality (up to 85 %) and high phoretic mite rates (up to 52 mites per hundred bees). The establishment of DWV in colonies occurred after that of *V. destructor* and the mite infestation rate had a significant effect on the virus prevalence and load. CBPV appeared only transiently throughout the surveys. The data showed that, in tropical colonies with permanent brood rearing, *V. destructor* and DWV can reach high levels, but are still subject to seasonal variations that appear to be influenced by environmental conditions. This suggests that beekeeping practices could be adapted by favouring sites and periods for transhumance or acaricide treatment.

1. Introduction

The parasitic mite *Varroa destructor* is one major biological threat to honeybees worldwide (Le Conte et al., 2010; Noël et al., 2020). It is an obligate parasite that completes its reproductive cycle on honeybee immature stages. It feeds on fat body tissues, weakening the honeybees and reducing their mass (Ramsey et al., 2019). It induces immunosuppression, facilitating the spread of many viruses, and may eventually reduce the life span of the insect (Noël et al., 2020; Traynor et al., 2020). *V. destructor* infestation greatly increases the prevalence and load of the Deformed Wing Virus (DWV) by providing efficient horizontal transmission routes (Ryabov et al., 2014a; Wilfert et al., 2016). Several DWV types have been distinguished. The titer and prevalence of the two main variants, DWV-A and DWV-B, are greatly enhanced by *V. destructor* infection. DW-B is biologically vectored by the *V. destructor*, i.e. it is capable of replicating in the mite's tissues, whereas DWV-A seems transmitted in a non-propagative matter, although one study showed that some of its genotypes are capable of replication in the mite (Damayo et al., 2023; Gisder and Genersch, 2021; Posada-Florez et al., 2019). Other parasite-independent, but less efficient, transmission routes are known, including trophallaxis, cannibalization or transovarian vertical transfer (Amiri et al., 2018; Chen et al., 2006). While the dynamics of *V. destructor* and its associated viruses have been documented in temperate climate, only a few studies focused on tropical areas where colonies are active all year long, *i.e.* without interruption of brood rearing (Martin et al., 2012).

In 2017, *V. destructor* invaded the tropical island of La Réunion (Esnault, 2019). This island is located in the South West Indian Ocean (Esnault, 2019), where *Apis mellifera unicolor*, an African lineage subspecies is endemic (Ruttner, 1988; Techer et al., 2017). Prior to arrival of

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V. destructor, the Chronic Bee Paralysis Virus (CBPV), the Black Queen Cell Virus (BQCV), the microsporidian *Nosema ceranae* and the European foulbrood agent *Mellisssococcus plutonius*, were reported, without significant impact on colonies (Esnault, 2019). DWV was detected only once in one colony in 2015.

The present study aimed to investigate the colony invasion and the seasonal dynamics of *V. destructor*, CBPV and DWV in the tropical island of La Réunion. As the altitude gradient of the island results in different microclimates and landscapes (Cadet, 1974), two apiaries of twenty colonies, one at high altitude and one at low altitude, were monitored monthly in order to identify possible seasonal patterns and parameters that could influence their dynamics.

2. Material and methods

2.1. Experimental design

Experiments were conducted in two apiaries located in the south of La Réunion, one at relatively high altitude (21° 17′ 3.426″S, 55° 33′ 41.819″E, 900 m asl), hereafter named H-A, and the other at low altitude (21° 19′ 19.107″S, 55° 29′ 20.156″E, 150 m asl), named L-A. The apiaries differed in their landscape (agricultural for H-A, suburban for L-A) and climate (colder and wetter for H-A). Forty colonies derived from eight ancestral queens were evenly separated in H-A and L-A apiaries (Supplementary Table 1). A first experiment was conducted from February 2019 to February 2020. For the second experiment, conducted from August 2020 to August 2021, sets of 20 colonies were reconstituted from new colonies and from the surviving colonies of the first experiment.

For both experiments, colonies were placed in 10-frame Langstroth hives and equalised for their brood content in order to homogenise their strength and potential pathogen loads. Colonies received a twelve-week treatment with amitraz (Apivar®) against *V. destructor*. Hives were inspected monthly, starting one month after amitraz strips removal. The colonies did not receive any further treatment, allowing requeening and collapse events.

2.2. Requeening and brood dynamic

The terminology "Requeening" included here reproductive swarming and supersedure events. Reproductive swarming was described by Winston (1980a) as the departure of the elder queen and a part of the workers from the colony for a new site. It differs from supersedure, which is the process of replacing an old queen by a new one in the colony without the departure of either queen or workers (Allen, 1965; Allsopp and Hepburn, 1997; Butler, 1957). The original tracked queens were colour-marked on their thorax, and one of their wings was clipped. A requeening event was recorded when the original queen was missing and replaced by another one.

The number of capped brood cells, chosen as a proxy of colony strength, was estimated monthly using the ColEval method (Hernandez et al., 2020; Maisonnasse et al., 2016) that consists in the visual evaluation of the proportion of capped brood surface relative to the total internal surface of a frame. Frames were also photographed and *ca.* 5 % (n = 260) of the pictures were randomly selected and analysed using the Image J software (version 1.53e) (Abràmoff, 2004) to perform an independent measurement of the capped brood proportion. The variation between the two methods was less than 5 %, validating the ColEval method.

2.3. Phoretic mite infestation rate

A phoretic mite refers to an adult *Varroa* that travels and probably parasitizes adult honeybees (White et al., 2017). Approximately 300 workers per colony were collected monthly from the centre of the brood nest and transferred in a 90 % ethanol solution. The mites were separated from the honeybees by three washes over a double sieve, one retaining the workers and the other the mites. The mites were counted and the infestation rate represented the number of *Varroa* mites per 100 bees, hereafter expressed in %. Samples were not taken when a few bees remained in the colony, *i.e.*, when the total colony population was restricted to two frames, in order to prevent artificial colony death.

2.4. DWV and CBPV loads

Twenty workers were sampled monthly on the flight board of each hive and placed in a 90 % ethanol solution. Samples were immediately stored on ice for less than 1 h before being transferred to −80 °C until RNA extraction. Sampling was not performed if there were not enough foragers at the hive entrance. The twenty workers were placed in a shredding bag (Bioreba) containing 2 mL of phosphate-buffered saline, and hand-shredded for 1 min using a manual homogeniser (Bioreba). Total RNA was extracted from 100 µL of the obtained lysate using the AllPrep DNA/RNA minikit (QIAGEN) and eluted in 60 µL of RNase-free water. RNA concentration was measured using the Qubit™ RNA high sensitivity (HS) Assay Kit (Thermo Fisher). Samples with RNA concentration below 2.8 $\mu g.mL^{-1}$ were discarded. Two hundred and forty-three RNA samples were analysed for the first-year experiment (138 and 105 from L-A and H-A respectively) and 284 for the second experiment (151 and 133 from L-A and H-A respectively).

The cDNA was synthesized using the ImProm-II™ Reverse Transcription System (PROMEGA) using 25 ng or 100 ng of RNA, 0.5 µg of random hexamers and 5 mM of $MgCl_2$. Following a hybridisation step for 5 min at 25 °C, primer extension was performed for 60 min at 42 °C, then the reverse transcriptase was inactivated at 70 °C for 10 min. The obtained cDNAs were diluted 20 times and stored at −20 °C. Negative controls were performed without reverse transcriptase.

Viral loads of DWV and CBPV were quantified by QPCR on an ABI PRISM 700 thermocycler (Applied Biosystems) using Fast Sybr Green MasterMix (Thermo Fisher) with 10 µL of diluted cDNA and 500 nM of each primer in a final volume of 20 µL. Primers 59 and 60 from Ryabov et al., (2014a) allowed the amplification of a DWV fragment common to both DWV-A and DWV-B. Primers for CBPV amplification were L67 and R467 from Cox-Foster et al. (2007). Reactions were performed in duplicates on MicroAmp Fast Optical 96-Well Reaction Plates (Applied Biosystems). The cycling parameters included a denaturation step at 95 °C for 20 s, 40 cycles with denaturation at 95 °C for 3 s and hybridisation and elongation at 60 °C for 30 s. Negative controls were included in each QPCR assay.

Plasmids carrying viral sequences that include the specific DWV and CBPV amplification products were built and quantified (Supplementary Materials). QPCR reactions were performed in every amplification run with a range of 10 to 10^{10} copies of plasmid in order to assess the linear relationship between the plasmid copy numbers, hereafter considered a genome equivalent (GE), and the Cycle threshold (C_T). Primer couples were validated for their amplification linearity and efficiency on both cDNA and plasmid matrices, ranging from 10^3 and 10^{10} GE copies and 10^2 and 10^{10} GE copies per reaction for DWV and CPBV, respectively. The viral loads were expressed as GE copies per µg of total honeybee RNA. Data of all negative controls were below the detection threshold.

2.5. Statistical analyses

Statistical analyses were performed using the R Studio software (Version 2022.7.1.554) (RStudio Team, 2015). Linear mixed effects models (LMMs) and generalised mixed models (GLMMs) were performed using the *lm* and *glmm* functions of the *lme4* package respectively. Models with the greatest explanatory power were chosen based on a combination of Akaike's Information Criterion (AIC) and analysis of variance (analysis of deviance in the GLMM case) using *anova* function. Models were followed by the analysis of variance (ANOVA), using the function *anova* included into the *car* package to compare the different

predictive variables. Pairwise comparisons of EMMeans were used as *post-hoc* tests and were performed by the function *emmeans* from the *emmeans* package.

To determine which variables influenced the phoretic mite infestation rates, only the data that followed the *V. destructor* installation were considered (*i.e.* starting from the fifth and third month in the first and second experiment respectively) and the following explanatory variables were included: experiment, sampling month, apiary location and capped brood surface.

Virus prevalence was analysed using GLMMs based on a binomial distribution, taking the phoretic mite infestation rate, the sampling month, the capped brood surface and the apiary location as explanatory variables. Virus load dynamics were performed on the log-transformed data of positive samples, taking as explanatory variables into LMM the phoretic mite infestation rate, the capped brood surface, the apiary location and the sampling month.

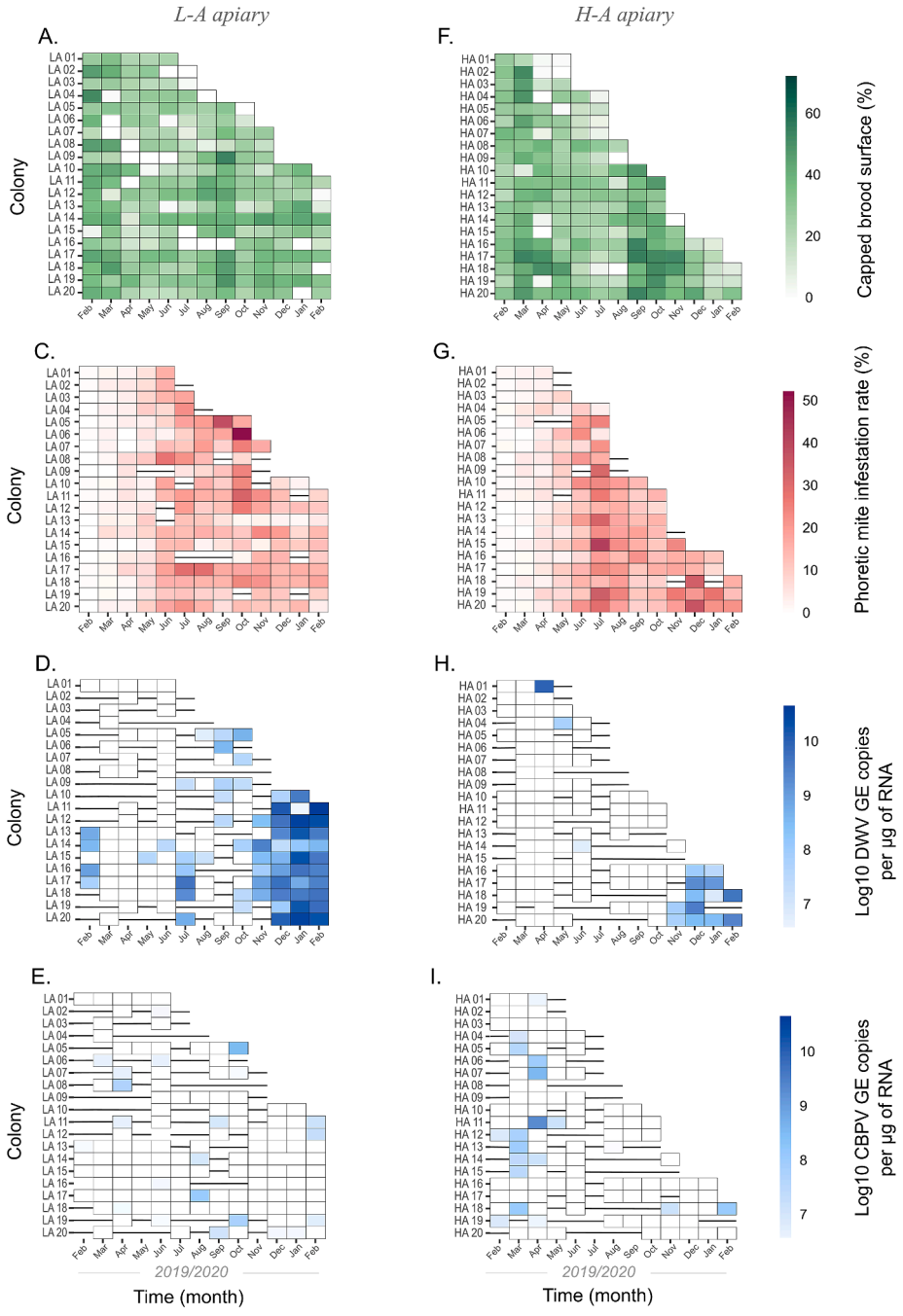

Fig. 1. First survey of colonies in apiaries located at low altitude (LA01 to LA20 colonies from L-A apiary, **A** to **D**) and high altitude (HA01 to HA20 colonies from H-A apiary, **E** to **H**) from Feb. 2019 to Feb. 2020 in La Réunion. The mean surface proportion occupied by capped brood cells (shaded green) is represented in **A** and **E**, the phoretic mite infestation rate (shaded red) in **B** and **F**, the *log*10 transformed of DWV and CBPV genome equivalent (GE) copies per µg of RNA (shaded blue) in **C**, **G** and **D**, **H** respectively. Colours change from light to dark with increasing values. Black lines and white rectangles indicate missing (no sampling) and null (no detection) values respectively. The cessation of data recording indicates the death of the colony. Consequently, the presence of data in the last time point indicates a colony that survived throughout the experiment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Colony mortality and phoretic mite infestation rate

In order to monitor the impact of *V. destructor* on colonies, no beekeeping measure was taken to prevent event such as absconding or swarming. Thus, colony collapse was not avoided and high mortalities were observed at the end of both experiments (Figs. 1 and 2). No kinship effect was observed between the colonies. The mortality was significantly higher in the H-A apiary (85 % and 75 % of the colonies for the first and the second experiments respectively) than in the L-A apiary (50 % and 55 %). Some colonies survived despite high phoretic mite infestation rates (up to 36.4 %), such as colonies 11, 12, 14, 17, 34, 38, 39 for the L-A apiary and colonies 18, 19, 20, 38 and 39 for the H-A apiary (Figs. 1B, F and 2B, F). Other colonies maintained relatively low phoretic mite infestation rates throughout the surveys such as colony 13 (below 12.4 %), 32 (below 6.4 %) for the L-A apiary and colony 40 (below 11.9 %) for the H-A apiary. In both apiaries and in both experiments, *V. destructor* managed to infest all colonies, two and one months after the removal of the acaricide strips in the first and the second experiments, respectively. The increase in the phoretic mite infestation rate in the colonies was initially exponential, with a doubling time of 1.0 ± 0.2 month (Supplementary Fig. S1). This exponential infestation phase had different durations in the two experimentations, from February to May and from August to September for the first and second experimentation respectively. Once established in the colonies, the

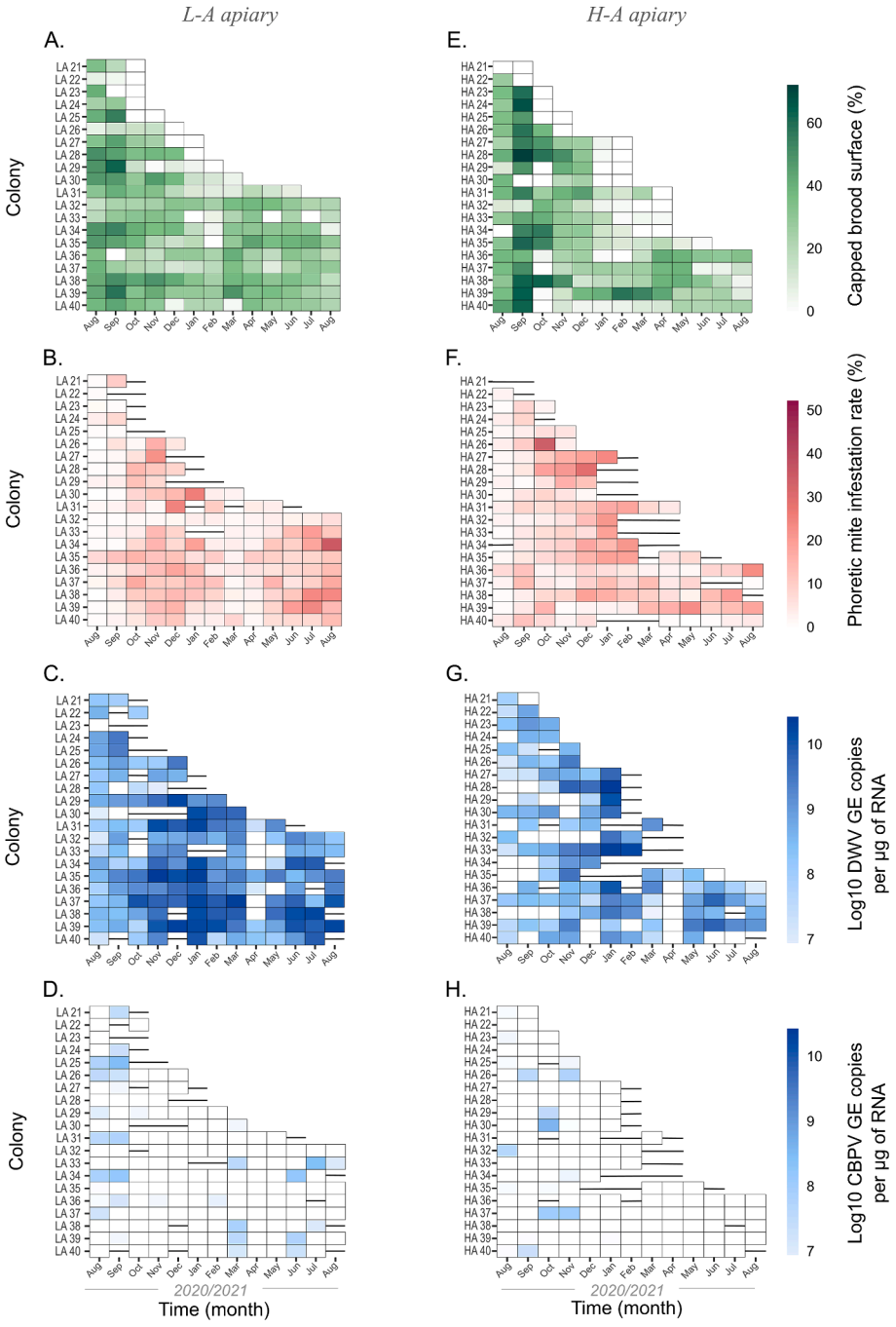

Fig. 2. Second survey of colonies in apiaries located at low altitude (LA21 to LA40 colonies from L-A apiary, **A** to **D**) and high altitude (HA21 to HA40 colonies from H-A apiary, **E** to **H**) from Aug. 2020 to Aug. 2021. Same legend as Fig. 1.

phoretic mite infestation rates varied from zero to 52 %. The experiment (ANOVA, F = 25.74, df = 1, p < 0.001), apiary location (ANOVA, F = 8.4, df = 1, p = 0.004) and sampling month (ANOVA, F = 4.74, df = 11, p < 0.001) significantly affected the infestation rates, but no significant effect was observed for the colony strength, *i.e.* the capped brood surface. While once *V. destructor* was installed there was no strong seasonal variation of the mite infestation rate from June in the first experiment, the rate significantly varied in the second experiment, especially in the L-A apiary, with high rates from November to January and July to August and with low rates from March to April. The highest and lowest mean infestation rates were especially significant over the second-year survey in the L-A apiary (Fig. 3, Supplementary Fig. S2). Significant interactions between the brood surface and the sampling month (ANOVA, F = 1.94, df = 11, p = 0.034), the experiment and the sampling month (ANOVA, F = 2.02, df = 8, p = 0.043), and the experiment and the apiary (ANOVA, F = 10.61, df = 1, p = 0.001) were found.

3.2. Requeening and brood dynamic

In both apiaries, capped brood was present throughout the year and never fell below 5 % of the frame area (Figs. 1A, E and 2A, E). Requeening events were observed throughout the year except in July-August and December-January, corresponding to periods when brood areas were smaller (Supplementary Fig. S3). The requeening events seemed to be consecutive to honey flow periods, showing a more contrasted dynamics in H-A, with high peaks in April and November 2019, and in September 2020 and May 2021.

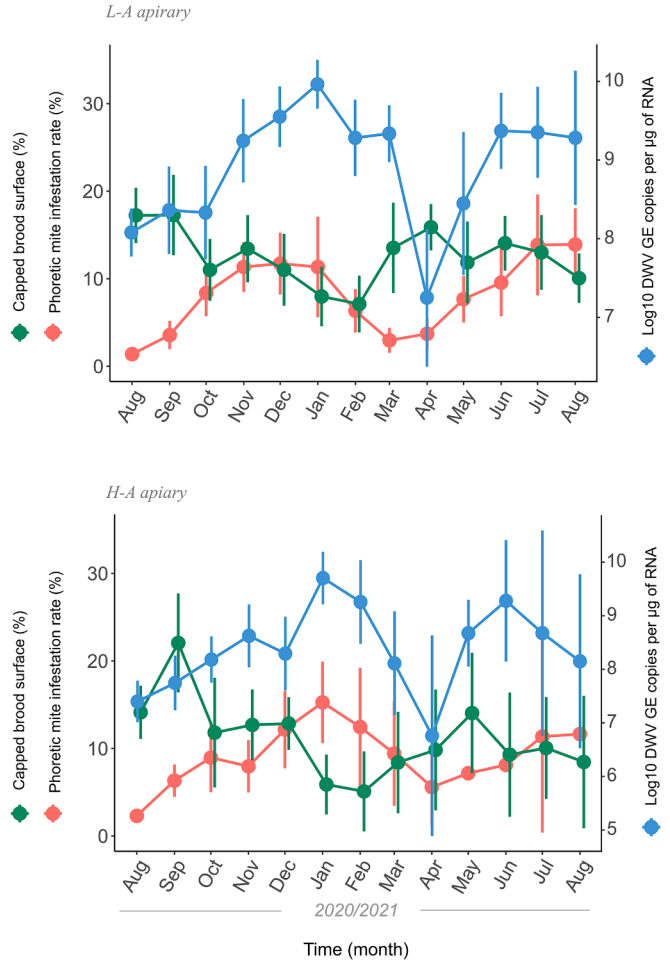

Fig. 3. Dynamics of brood, *V. destructor* infestation and DWV load in apiaries located at low altitude (L-A apiary) and high altitude (H-A apiary) from Aug. 2020 to Aug. 2021. Values represent the means of capped brood surface (green), phoretic mite infestation rate (red) and *log*10 transformed of DWV genome equivalent (GE) copies per µg of RNA (blue). Bars indicate the confidence intervals (α = 0,05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Establishment and persistence of DWV

In the first experiment, DWV was detected in 51.4 % of the L-A samples and in 17.1 % of the H-A samples. A progressive establishment of DWV in the colonies was observed in both L-A and H-A apiaries. During the first 9 months, only 16 out of 40 colonies were positive for DWV at least once. At the end of the experiment, all colonies carried the virus (Figs. 1C, G and 2C, G).

In the first experiment, apiary location and sampling month had a significant effect on DWV prevalence (χ^2 = 5.80; p = 0.016 and χ^2 = 122.7; p < 0.001, respectively). The interactions between infestation rate and colony strength (χ^2 = 5. 46; p = 0.019), apiary location and colony strength (χ^2 = 144.17; p < 0.001), and colony strength and sampling month (χ^2 = 30.53; p 0.004) were also significant. Finally, a significant second-order interaction was observed between apiary location, sampling month and colony strength (χ^2 = 770.75; p < 0.001).

In the second experiment DWV was detected in almost all RNA samples (92.7 % in L-A and 75.9 % in H-A) and all colonies carried the virus. Although there was a temporary lack of detection, the colonies did not get rid of the virus. DWV prevalence was significantly higher in L-A than in H-A (χ^2 = 20.31; p < 0.001), and sampling (χ^2 = 28.78; p = 0.004) and phoretic mite infestation (χ^2 = 10; p = 0.002) had a significant effect on DWV prevalence.

DWV loads ranged from 10^6 to 10^{10} GE copies per µg of total RNA during the first experiment, with the highest loads observed at the end of the monitoring period (November to February). The magnitude of the DWV loads was the same in the second experiment, but higher loads were observed throughout the monitoring period. Only in the second experiment did DWV loads significantly increase with the phoretic mite infestation rate (χ^2 = 9.15; p = 0.002). The sampling month also had a significant effect on the DWV load (χ^2 = 82.2; p < 0.001). DWV loads were also significantly higher in L-A than in H-A (χ^2 = 13.4; p < 0.001).

An increase in the average DWV load and an increase in the average phoretic mite infestation rate were observed in the first months of monitoring in the second survey (Fig. 3). However, a decrease in average DWV load was observed in April in both apiaries, but only significant in the L-A apiary (Supplementary Fig. S2).

3.4. CBPV occurred transiently

In contrast to DWV, CBPV was detected in only 18.5 % of the samples in the first experiment (18.1 % in L-A and 19.0 % in H-A, respectively) and in 19.0 % of the samples in the second experiment (22.5 % in L-A and 15.0 % in H-A) (Fig. 1D, F and 2D, 2H). CBPV prevalence was not correlated with any explanatory variables, and the presence of the virus was transient.

Measured CBPV loads ranged from 10^6 to 10^9 GE copies per µg of total RNA in the first experiment, and from 10^5 to 10^8 in the second experiment. There was no significant relationship between CBPV loads and the variables tested in either experiment.

4. Discussion

Populations of *A. m. unicolor* reared brood permanently and requeening occurred throughout the year, as previously observed for *A. m. scutellata* and *A. m. jemenitica* of the African lineage in warm climates (Al-Ghamdi et al., 2017; Schneider and Blyther, 1988) and for Africanised honeybees in tropical climates (Winston, 1980). The population dynamics of honeybees in La Réunion seemed to be driven by resource availability rather than by seasonal changes. The increase in brood area corresponded to the two main honey flows of *Schinus terebenthifolius*

from March to April and *Litchi sinensis* from August to September (Esnault et al., 2014), with a lag between H-A and L-A sites due to the flowering lag time at high altitude. Other less substantial honey flows occur throughout the year depending on the location on the island (Esnault et al., 2014), providing regular resources that could sustain brood production and potential swarming throughout the year. In *A. m. scutellata* in Botswana, a positive correlation between foraging activity and brood abundance has been observed (Schneider and McNally, 1992).

The two-year study revealed a seasonal dynamic pattern of phoretic mite infestation rate, the establishment and persistence of DWV in all colonies, whereas CBPV was only transient. In both experiments the initial increase of *V. destructor* infestation was exponential but for a shorter period in the second experiment. On the one hand, this could be explained by a higher initial rate of phoretic mites in the colonies at the beginning of the second experiment compared to the first one (Supplementary Fig. S1). This higher rate could be related to a less efficient amitraz treatment against *V. destructor* or to the invasive spread of the mite in the environment through time. On the other hand, the significantly lower level of infestation at the end of the exponential increase in the second experiment (8.7 ± 6.4 % *vs* 14.8 ± 5.4 % in the first experiment, emmeans; $p < 0.001$; Supplementary Fig. S1) could also explain its shorter period. The cessation of the exponential infestation could be due to limiting factors that could have differed between the experiments, and that could be related to the honeybee biology (*e.g.* brood quantity, adult worker population, resistance to the mite) or to the colony environment.

Throughout the surveys following this initial increase, the mean mite infestation rate was 10.3 ± 8.7 % in the first experiment (starting from May 2019), reaching up to 52 %, and 8.0 ± 6.6 % bees in the second experiment (from October 2020). It was significantly lower in the second experiment (Mann-Whitney $p = 0.006$). These rates were higher than those observed in colonies of Africanised honeybees in Latin America, with mean infestation rates ranging from 3.5 % to 9 %, or in colonies of *A. m. scutellata* in South Africa and in Kenya, with rates below 4 % (Anguiano-Baez et al., 2016; Cheruiyot et al., 2020; Maggi et al., 2016; Medina Flores et al., 2014; Tibatá et al., 2021). Some authors consider rates above 3 % to be high (Giacobino et al., 2016). Interestingly, similar high levels of infestation have been observed in colonies exposed to *V. destructor* for two years in New Zealand, with a median rate over 10 % (Mondet et al., 2014). The high rates observed in the present work may therefore reflect the invasive phase of *V. destructor*. Such a rapid increase in population ought to subsequently decrease (Gurevitch et al., 2011), as observed with lower infestation rates in New Zealand several years after invasion (Mondet et al., 2014). This demographic decline could be due to host-parasite co-evolution, as suggested by the selection of resistance traits in some honeybee subspecies (Guichard et al., 2020) and by the population structure of *V. destructor* in Europe (Moro et al., 2021). The continuous survey of *V. destructor* population over the next years may show if such demographic changes also occur in a tropical island".

The significant effect observed of the sampling month, with an interaction with brood area on *V. destructor* infestation rate could be related to resource availability. The quantity and quality of resource are thought to affect the honeybee immunity (Alaux et al., 2010; Pasquale et al., 2013), and colony strength (capped brood surface, adult population, etc.) (Ochungo et al., 2022), which in turn could affect the mite reproduction and population. Here, the brood was reared continuously (Figs. 1 and 2), but the capped brood surface alone did not significantly affect *V. destructor* infestation, once installed in the colonies. However, in *A. m. scutellata* the mite density can be positively associated with brood and imago densities (Cheruiyot et al., 2020). While the continuous presence of the brood may be involved in the high initial *Varroa* infestation, it did not explain the subsequent variation in parasite infestation. For instance, honeybees in the L-A apiary were significantly less infested by *Varroa* than in the H-A apiary. Regional or climatic influence on *Varroa* infestation has already been demonstrated in previous studies (Tibatá et al., 2021; Invernizzi et al., 2011). This suggests that the environmental conditions of the colonies, such as landscape and climate, may influence the expression of resistant traits by the honeybee and thus the reproductive success of *V. destructor* (Mondet et al., 2020).

Prior to the introduction of *V. destructor* in La Réunion, DWV had only been PCR-detected once in 2015. During the first few months of the first experiment most of the colonies were DWV-free, but by the end of the monitoring period, all were infected with high viral loads (Figs. 1 and 2). These results suggest that the development of *V. destructor* gradually led to the establishment of DWV in the colonies. Establishment of *V. destructor* and DWV did not occur simultaneously, with high virus prevalence and loads occurring four to five months after the exponential establishment of *V. destructor*. The significant interaction between the sampling month and the apiary location on the virus prevalence may be due to an earlier occurrence of DWV in the L-A apiary, but also to the higher mortality rate in the H-A apiary, which reduced the number of colonies monitored (Fig. 1). The association between *V. destructor* and the DWV prevalence and loads has well been documented, including studies in insular territories on European honeybee lineages (Martin et al., 2012; Mondet et al., 2014). As in the present work, *V. destructor* invasion in Hawaii resulted in 100 % DWV prevalence in colonies, with loads of about 10^{10} copies per honeybee after three years. At the beginning of the second experiment, despite the 12-week treatment that almost eradicated *V. destructor*, all colonies were already DWV-positive, showing the persistence of the virus in a worker generation reared without *V. destructor*. DWV is not exclusively transmitted by *V. destructor*. It can also spread and persist in a mite free colony by other horizontal and vertical transmission routes (Amiri et al., 2018; Chen et al., 2006).

In the second experiment, both DWV prevalence and load were significantly influenced by the *Varroa* mite infestation rate, the apiary location and the sampling month (Fig. 3 and Supplementary Fig. S2). The DWV load was positively associated with the phoretic *Varroa* mite rate. It has been shown that viral loads of both DWV-A and DWV-B are higher in *Varroa* infested colonies than in non-infested colonies and that there is a positive correlation between mite and DWV loads (Norton et al., 2021). However, in contrast to *V. destructor* rates, the DWV load and prevalence were significantly higher in the L-A apiary than in the H-A apiary, showing that not only the mite but also environmental conditions can affect the virus replication.

A significant and sharp decrease in DWV load occurred in both apiaries in April 2021, corresponding to the flowering of *Schinus terebinthifolius* and the main honey flow on the island (Fig. 3). Two non-exclusive hypotheses could link these two events. Firstly, the honey flow may have caused an increase in the brood area, followed by an increase in the adult worker population, reducing the DWV and mite-to-honeybee ratios. Second, it has been shown that higher resource quality can enhance the honeybee immunity and attenuate the effect of *V. destructor* on pupae weight loss (Alaux et al., 2010; Pasquale et al., 2013; Piou et al., 2018). Here, the honey flow may thus have improved the honeybee immunity, leading to a reduction in DWV loads. Noteworthy, such a reduction in DWV load was not observed in the first experiment, as the virus was not yet established in the apiaries at that time.

As observed in New Zealand, the establishment of *V. destructor* did not increase CBPV prevalence and load (Mondet et al., 2014). This lack of relationship between CBPV and *Varroa* has been previously documented (Ball and Allen, 1988; Chantawannakul et al., 2006; Ribière et al., 2010; Tentcheva et al., 2004). CBPV may not be transmitted by *Varroa,* but rather by repeated contact between healthy and infected individuals or by faeces (Al Naggar and Paxton, 2020; Ribière et al., 2010, 2007).

At the end of both experiments, high colony mortality was observed in both apiaries, with no obvious pattern. Mortality was higher in the H-A apiary, which also had the highest rate of mite infestation. This could

be explained by the high-altitude environment, which offers lower temperatures and scarcer resources than the low-altitude environment. Colony mortality started as soon as *V. destructor* was established in the colonies (i.e., after the exponential phase) and reached 50 to 85 % of colony losses after one year depending on the apiary and the experiment. For comparison, there was no mortality during the 3-month treatment prior to the experiments and the annual mortality before the arrival of *V. destructor* on the island was less than 1 % (Esnault, 2019), confirming *V. destructor* as the driver of colony mortality. The African *A. m. capensis* and *A. m. scutellata* or the Africanised honeybee populations are known to be more tolerant or even resistant to *V. destructor* than European subspecies (Camazine, 1986; Guzmán-Novoa et al., 1999; Martin and Medina, 2004). Nevertheless, these honeybee populations have been in contact with the mite for over thirty years (Wilfert et al., 2016). The high mortality observed in La Réunion suggests that, despite belonging to the African lineage, *A. m. unicolor* honeybees lack such tolerance/resistance traits and/or were naive to *V. destructor*, *i.e.* they had not yet been counter-selected, and showed higher sensitivity to the mite.

This study confirmed the link between *V. destructor* invasion and the establishment and persistence of DWV. The dynamics of these pathogens could also be influenced by a number of interdependent biotic and abiotic factors such as colony strength, altitude, resource availability, temperature… Thus, beekeeping practices in areas of high landscape and climate diversity such as La Réunion could be adapted, for example by favouring or avoiding sites and periods for transhumance or by optimising the period for acaricide treatment. Moreover, there were 15 % to 50 % of colonies that survived the experiments despite high mite and DWV loads or that maintained relatively low mite and DWV loads, suggesting potential traits of the resistance and tolerance to pathogens.

CRediT authorship contribution statement

Benoit Jobart: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation. **Hélène Delatte:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Gérard Lebreton:** Methodology, Investigation, Conceptualization. **Nicolas Cazanove:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation. **Olivier Esnault:** Writing – review & editing, Validation, Supervision, Methodology. **Johanna Clémencet:** Writing – review & editing, Supervision, Methodology. **Nicolas Blot:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2024.108125.

References

Abràmoff, M.D., 2004. Image Processing with ImageJ 7.

Al Naggar, Y., Paxton, R.J., 2020. Mode of transmission determines the virulence of Black Queen Cell Virus in adult honey bees, posing a future threat to bees and apiculture. Viruses 12, 535. https://doi.org/10.3390/v12050535.

Alaux, C., Ducloz, F., Crauser, D., Le Conte, Y., 2010. Diet effects on honeybee immunocompetence. Biol. Lett. 6, 562–565. https://doi.org/10.1098/rsbl.2009.0986.

Al-Ghamdi, A.A., Adgaba, N., Tadesse, Y., Getachew, A., Al-Maktary, A.A., 2017. Comparative study on the dynamics and performances of *Apis mellifera jemenitica* and imported hybrid honeybee colonies in southwestern Saudi Arabia. Saudi Journal of Biological Sciences, Current Research in Apiculture 24, 1086–1093. https://doi.org/10.1016/j.sjbs.2017.01.008.

Allen, M.D., 1965. The production of queen cups and queen cells in relation to the general development of honeybee colonies, and its connection with swarming and supersedure. J. Apic. Res. 4, 121–141. https://doi.org/10.1080/00218839.1965.11100115.

Allsopp, M.H., Hepburn, H.R., 1997. Swarming, supersedure and the mating system of a natural population of honey bees (*Apis mellifera capensis*). J. Apic. Res. 36, 41–48. https://doi.org/10.1080/00218839.1997.11100929.

Amiri, E., Kryger, P., Meixner, M.D., Strand, M.K., Tarpy, D.R., Rueppell, O., 2018. Quantitative patterns of vertical transmission of deformed wing virus in honey bees. PLoS One 13, e0195283. https://doi.org/10.1371/journal.pone.0195283.

Anguiano-Baez, R., Guzman-Novoa, E., Hamiduzzaman, M., Espinosa-Montaño, L.G., Correa-Benítez, A., 2016. *Varroa destructor* (Mesostigmata: Varroidae) parasitism and climate differentially influence the prevalence, levels, and overt infections of Deformed Wing Virus in honey bees (Hymenoptera: Apidae). J. Insect Sci. 16, 44. https://doi.org/10.1093/jisesa/iew029.

Ball, B.V., Allen, M.F., 1988. The prevalence of pathogens in honey bee (*Apis mellifera*) colonies infested with the parasitic mite *Varroa jacobsoni*. Ann. Appl. Biol. 113, 237–244. https://doi.org/10.1111/j.1744-7348.1988.tb03300.x.

Butler, C.G., 1957. The process of queen supersedure in colonies of honeybees (*Apis mellifera* Linn.). Ins. Soc 4, 211–223. https://doi.org/10.1007/BF02222154.

Cadet, Th., 1974. Étude sur la végétation des hautes altitudes de l'île de la Réunion (Océan Indien). Plant Ecol 29, 121–130. https://doi.org/10.1007/BF02389717.

Camazine, S., 1986. Differential reproduction of the mite, *Varroa jacobsoni* (Mesostigmata: Varroidae), on Africanized and European honey bees (Hymenoptera: Apidae). Annals of the Entomological Society of America 79, 801–803. https://doi.org/10.1093/aesa/79.5.801.

Chantawannakul, P., Ward, L., Boonham, N., Brown, M., 2006. A scientific note on the detection of honeybee viruses using real-time PCR (TaqMan) in Varroa mites collected from a Thai honeybee (*Apis mellifera*) apiary. J. Invertebr. Pathol. 91, 69–73. https://doi.org/10.1016/j.jip.2005.11.001.

Chen, Y., Evans, J., Feldlaufer, M., 2006. Horizontal and vertical transmission of viruses in the honey bee, *Apis mellifera*. Journal of Invertebrate Pathology, Society of Invertebrate Pathology 2006 Special Issue 92, 152–159. https://doi.org/10.1016/j.jip.2006.03.010.

Cheruiyot, S.K., Kahuthia-Gathu, R., Mbugi, J.P., Muli, E., Lattorff, H.M.G., 2020. Population abundance of *Varroa destructor* and its effects on *Apis mellifera scutellata* colonies in Kenya. Exp Appl Acarol 82, 171–184. https://doi.org/10.1007/s10493-020-00548-5.

Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., Quan, P.-L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., vanEngelsdorp, D., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchison, S.K., Simons, J.F., Egholm, M., Pettis, J.S., Lipkin, W.I., 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. Science 318, 283–287. https://doi.org/10.1126/science.1146498.

Damayo, J.E., McKee, R.C., Buchmann, G., Norton, A.M., Ashe, A., Remnant, E.J., 2023. Virus replication in the honey bee parasite, *Varroa destructor*. J. Virol. 97.

Esnault, O., Sinelle, J., Lesquin, S., Reynaud, B., Delatte, H., 2014. Caractérisation de l'apiculture réunionnaise : chiffres-clés, pratiques et typologie. La Santé De L'abeille 325–344.

Esnault, O., 2019. Diversité des agents pathogènes de l'abeille dans le Sud-Ouest de l'Océan Indien dans un contexte d'invasion récente de *Varroa destructor* et mortalités associées.

Giacobino, A., Molineri, A., Bulacio Cagnolo, N., Merke, J., Orellano, E., Bertozzi, E., Masciangelo, G., Pietronave, H., Pacini, A., Salto, C., Signorini, M., 2016. Key management practices to prevent high infestation levels of *Varroa destructor* in honey bee colonies at the beginning of the honey yield season. Prev. Vet. Med. 131, 95–102. https://doi.org/10.1016/j.prevetmed.2016.07.013.

Gisder, S., Genersch, E., 2021. Direct evidence for infection of *Varroa destructor* mites with the bee-pathogenic deformed wing virus variant B, but not variant A, via Fluorescence *in situ* hybridization analysis. J Virol 95. https://doi.org/10.1128/JVI.01786-20.

Guichard, M., Dietemann, V., Neuditschko, M., Dainat, B., 2020. Advances and perspectives in selecting resistance traits against the parasitic mite *Varroa destructor* in honey bees. Genet. Sel. Evol. 52, 1–22.

Gurevitch, J., Fox, G.A., Wardle, G.M., Inderjit Taub, D., 2011. Emergent insights from the synthesis of conceptual frameworks for biological invasions. Ecol. Lett. 14, 407–418. https://doi.org/10.1111/j.1461-0248.2011.01594.x.

Guzmán-Novoa, E., Vandame, R., Arechavaleta, M.E., 1999. Susceptibility of European and Africanized honey bees (*Apis mellifera* L.) to Varroa jacobsoni Oud. in Mexico. Apidologie 30, 173–182. https://doi.org/10.1051/apido:19990207.

Hernandez, J., Maisonnasse, A., Cousin, M., Beri, C., Le Quintrec, C., Bouetard, A., Castex, D., Decante, D., Servel, E., Buchwalder, G., Brunet, F., Feschet-Destrella, E., de Bellescize, K., Kairo, G., Frontero, L., Pédehontaa-Hiaa, M., Buisson, R., Pouderoux, T., Aebi, A., Kretzschmar, A., 2020. ColEval: honeybee COLony structure

evaluation for Field surveys. Insects 11, 41. https://doi.org/10.3390/insects11010041.

Invernizzi, C., Santos, L.E., García, E., Daners, G., Di Landro, R., Saadoun, A., Cabrera, Vique C., 2011. Sanitary and nutritional characterization of honeybee colonies in eucalyptus grandis plantations. Archivos de zootecnia. 60 (232), 1303–1314.

Le Conte, Y., Ellis, M., Ritter, W., 2010. *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? Apidologie 41, 353–363. https://doi.org/10.1051/apido/2010017.

Maggi, M., Antúnez, K., Invernizzi, C., Aldea, P., Vargas, M., Negri, P., Brasesco, C., De Jong, D., Message, D., Teixeira, E.W., Principal, J., Barrios, C., Ruffinengo, S., Da Silva, R.R., Eguaras, M., 2016. Honeybee health in South America. Apidologie 47, 835–854. https://doi.org/10.1007/s13592-016-0445-7.

Maisonnasse, A., Hernandez, J., Le Quintrec, C., M, C., Beri, C., Kretzschmar, A., 2016. Evaluation de la structure des colonies d'abeilles, création et utilisation de la méthode ColEval (Colony Evaluation). Doi: 10.15454/1.51358572217001E12.

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, 1304–1306. https://doi.org/10.1126/science.1220941.

Martin, S.J., Medina, L.M., 2004. Africanized honeybees have unique tolerance to *Varroa* mites. Trends Parasitol. 20, 112–114. https://doi.org/10.1016/j.pt.2004.01.001.

Medina Flores, C.A., Guzmán Novoa, E., Hamiduzzaman, M., Aréchiga Flores, C.F., López Carlos, M.A., 2014. Africanized honey bees (*Apis mellifera*) have low infestation levels of the mite *Varroa destructor* in different ecological regions in Mexico. Genetics and Molecular Research, Vol. 13, No. 3, 2014, 7282-7293.

Mondet, F., de Miranda, J.R., Kretzschmar, A., Le Conte, Y., Mercer, A.R., 2014. On the Front Line: quantitative virus dynamics in honeybee (*Apis mellifera* L.) colonies along a new expansion front of the parasite *Varroa destructor*. PLoS Pathog 10, e1004323. https://doi.org/10.1371/journal.ppat.1004323.

Mondet, F., Beaurepaire, A., McAfee, A., Locke, B., Alaux, C., Blanchard, S., Danka, B., Le Conte, Y., 2020. Honey bee survival mechanisms against the parasite *Varroa destructor*: a systematic review of phenotypic and genomic research efforts. Int. J. Parasitol. 50, 433–447. https://doi.org/10.1016/j.ijpara.2020.03.005.

Moro, A., Blacquière, T., Dahle, B., Dietemann, V., Le Conte, Y., Locke, B., Neumann, P., Beaurepaire, A., 2021. Adaptive population structure shifts in invasive parasitic mites, *Varroa destructor*. Ecol. Evol. 11, 5937–5949. https://doi.org/10.1002/ece3.7272.

Noël, A., Le Conte, Y., Mondet, F., 2020. *Varroa destructor*: how does it harm *Apis mellifera* honey bees and what can be done about it? Emerging Topics in Life Sciences 4, 45–57. https://doi.org/10.1042/ETLS20190125.

Norton, A.M., Remnant, E.J., Tom, J., Buchmann, G., Blacquiere, T., Beekman, M., 2021. Adaptation to vector-based transmission in a honeybee virus. J Anim Ecol 90, 2254–2267. https://doi.org/10.1111/1365-2656.13493.

Ochungo, P., Veldtman, R., Abdel-Rahman, E.M., Muli, E., Ng'ang'a, J., Tonnang, H.E.Z., Landmann, T., 2022. Fragmented landscapes affect honey bee colony strength at diverse spatial scales in agroecological landscapes in Kenya. Ecol. Appl. 32, e02483 https://doi.org/10.1002/eap.2483.

Pasquale, G.D., Salignon, M., Conte, Y.L., Belzunces, L.P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J.-L., Alaux, C., 2013. Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter? PLoS One 8, e72016. https://doi.org/10.1371/journal.pone.0072016.

Piou, V., Tabart, J., Hemptinne, J.-L., Vétillard, A., 2018. Effect of pollen extract supplementation on the varroatosis tolerance of honey bee (*Apis mellifera*) larvae reared in vitro. Exp Appl Acarol 74, 25–41. https://doi.org/10.1007/s10493-017-0198-7.

Posada-Florez, F., Childers, A.K., Heerman, M.C., Egekwu, N.I., Cook, S.C., Chen, Y., Evans, J.D., Ryabov, E.V., 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 USA 116, 1792–1801. https://doi.org/10.1073/pnas.1818371116.

Ribière, M., Lallemand, P., Iscache, A.-L., Schurr, F., Celle, O., Blanchard, P., Olivier, V., Faucon, J.-P., 2007. Spread of infectious Chronic Bee Paralysis Virus by honeybee (*Apis mellifera* L.) feces. Appl. Environ. Microbiol. 73, 7711–7716. https://doi.org/10.1128/AEM.01053-07.

Ribière, M., Olivier, V., Blanchard, P., 2010. Chronic bee paralysis: a disease and a virus like no other? J. Invertebr. Pathol. 103, S120–S131. https://doi.org/10.1016/j.jip.2009.06.013.

RStudio Team, 2015. RStudio | Open source & professional software for data science teams. URL https://www.rstudio.com/ (accessed 2.23.22).

Ruttner, F., 1988. Biogeography and Taxonomy of Honeybees. Springer Science & Business Media.

Ryabov, E.V., Wood, G.R., Fannon, J.M., Moore, J.D., Bull, J.C., Chandler, D., Mead, A., Burroughs, N., Evans, D.J., 2014. A Virulent strain of Deformed Wing Virus (DWV) of honeybees (*Apis mellifera*) prevails after *Varroa destructor*-mediated, or in vitro, transmission. PLoS Pathog. 10, e1004230 https://doi.org/10.1371/journal.ppat.1004230.

Schneider, S., Blyther, R., 1988. The habitat and nesting biology of the African honey bee *Apis mellifera scutellata* in the Okavango River Delta, Botswana. Africa. Ins. Soc 35, 167–181. https://doi.org/10.1007/BF02223930.

Schneider, S.S., McNally, L.C., 1992. Seasonal patterns of foraging activity in colonies of the African honey bee, *Apis mellifera scutellata*, in Africa. Ins. Soc 39, 181–193. https://doi.org/10.1007/BF01249293.

Techer, M.A., Clémencet, J., Simiand, C., Preeaduth, S., Azali, H.A., Reynaud, B., Delatte, H., 2017. Large-scale mitochondrial DNA analysis of native honey bee *Apis mellifera* populations reveals a new African subgroup private to the South West Indian Ocean islands. BMC Genet 18, 53. https://doi.org/10.1186/s12863-017-0520-8.

Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M.E., Bergoin, M., 2004. Prevalence and seasonal variations of six bee viruses in *Apis mellifera* L. and *Varroa destructor* mite populations in France. Appl. Environ. Microbiol. 70, 7185–7191. https://doi.org/10.1128/AEM.70.12.7185-7191.2004.

Tibatá, V.M., Sanchez, A., Palmer-Young, E., Junca, H., Solarte, V.M., Madella, S., Ariza, F., Figueroa, J., Corona, M., 2021. Africanized honey bees in Colombia exhibit high prevalence but low level of infestation of *Varroa* mites and low prevalence of pathogenic viruses. PLoS One 16, e0244906. https://doi.org/10.1371/journal.pone.0244906.

Traynor, K.S., Mondet, F., de Miranda, J.R., Techer, M., Kowallik, V., Oddie, M.A.Y., Chantawannakul, P., McAfee, A., 2020. *Varroa destructor*: a complex parasite, crippling honey bees worldwide. Trends Parasitol. 36, 592–606. https://doi.org/10.1016/j.pt.2020.04.004.

White, P.S., Morran, L., de Roode, J., 2017. Phoresy. Curr. Biol. 27, R578–R580. https://doi.org/10.1016/j.cub.2017.03.073.

Wilfert, L., Long, G., Leggett, H.C., Schmid-Hempel, P., Butlin, R., Martin, S.J.M., Boots, M., 2016. Deformed wing virus is a recent global epidemic in honeybees driven by *Varroa* mites. Science 351, 594–597. https://doi.org/10.1126/science.aac9976.

Winston, M.L., 1980a. Swarming, afterswarming, and reproductive rate of unmanaged honeybee colonies (*Apis mellifera*). Ins. Soc 27, 391–398. https://doi.org/10.1007/BF02223731.

Winston, M.L., 1980b. Seasonal patterns of brood rearing and worker longevity in colonies of the Africanized honey bee (Hymenoptera: Apidae) in South America. J. Kansas Entomol. Soc. 53, 157–165.