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Supplementary Materials

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Global Honey Bee Viral Landscape Altered by a Parasitic Mite

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Emerging diseases are among the greatest threats to honey bees. Unfortunately, where and when an emerging disease will appear are almost impossible to predict. The arrival of the parasitic *Varroa* mite into the Hawaiian honey bee population allowed us to investigate changes in the prevalence, load, and strain diversity of honey bee viruses. The mite increased the prevalence of a single viral species, deformed wing virus (DWV), from ~10 to 100% within honey bee populations, which was accompanied by a millionfold increase in viral titer and a massive reduction in DWV diversity, leading to the predominance of a single DWV strain. Therefore, the global spread of *Varroa* has selected DWV variants that have emerged to allow it to become one of the most widely distributed and contagious insect viruses on the planet.

The emergence of infectious diseases is driven largely by socioeconomic, environmental, and ecological factors (*1*), and these diseases have significant effects on biodiversity, agricultural biosecurity, global economies, and human health (*2*, *3*). The honey bee is one of the most economically important insects, providing crop pollination services and valuable hive products (*4*). During the past 50 years, the global spread of the ectoparasitic mite *Varroa destructor* has resulted in the death of millions of honey bee (*Apis mellifera*) colonies (*5*). There is general consensus that the mites' association with a range of honey bee RNA viruses is a contributing factor in the global collapse of honey bee colonies (*5*–*10*), because the spread of mites has facilitated the spread of viruses (*11*, *12*) by acting as a viral reservoir and incubator (*13*). In addition, the mites' feeding behavior allows virus to be transmitted directly into the bees' hemolymph, thus bypassing conventional, established oral and sexual routes of transmission. In particular, deformed wing virus (DWV) has been associated with the collapse of *Varroa*-infested honey bee colonies (*5*, *8*, *14*–*16*), because it is ubiquitous in areas where *Varroa* is well established (*6*, *9*, *17*, *18*). The rapid global spread of *Varroa* means that very little is known about the natural prevalence, viral load, and strain diversity of honey bee viruses before the *Varroa* invasion (*15*). Such data are important, because most honey bee viral infections were considered harmless before the spread of *Varroa* (*9*). Large-scale loss of honey bee colonies has been associated with viruses vectored by *Varroa* (*5*). The recent arrival and subsequent spread of *Varroa* across parts of the Hawaiian archipelago has provided an opportunity to study the initial phase of the evolution of the honey bee–*Varroa*–DWV association. So far, colony collapse disorder (CCD) (*6*) has not been reported in Hawaii (*19*), but all of the associated pests and pathogens are present.

European honey bees (*Apis mellifera* L.) were first introduced to Hawaii from California in 1857. They were largely managed, but feral populations were soon established on every major island in the archipelago (*20*). Hawaii remained *Varroa*-free until August 2007, when the mite was discovered throughout Oahu Island. A subsequent survey by S. Nikaido and E. Villalobos during 2007–2008 recorded the collapse of 274 of 419 untreated colonies belonging to beekeepers. The disappearance of feral colonies from urban areas on Oahu was also noticed by beekeepers and pest control officers. Despite quarantine measures, the mite spread to Hilo on the Big Island in January 2009, where it survived an eradication attempt and by November 2009 had spread throughout the southern region of the island (Fig. 1). By November 2010, *Varroa* occurred throughout the Big Island. However, the islands of Kauai and Maui remained mite-free, and no unusual colony losses or disease problems have been reported there (*19*). The aim of this study was to investigate the influence that *Varroa* has in the spread of honey bee viruses during the initial

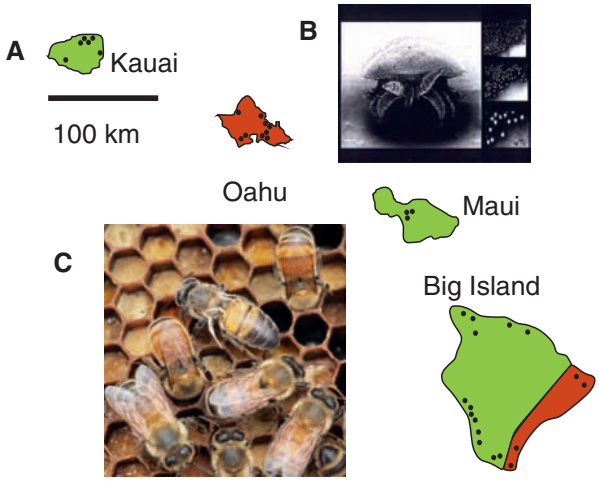

Fig. 1. (**A**) The four main Hawaiian Islands, showing the distribution of *Varroa* during 2009. Green and brown indicate *Varroa*-free and *Varroa* infested areas respectively. Dots indicate the location of each study apiary. By November 2010, *Varroa* was present throughout the Big Island. The co-occurrence of the *Varroa* mite (**B**) and DWV can result in overt symptoms of (**C**) deformed wings in honey bees, although many nondeformed bees also carry high DWV loads.

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phase of establishment. The spread of *Varroa* is normally from point introductions characteristic of pest species, so the arrival and spread of the mite across Hawaii are typical for this species.

In 2009, our study of 293 honey bee colonies, from 35 apiaries on the four main Hawaiian Islands (Fig. 1), revealed that the exposure to *Varroa* had a significant effect on the prevalence, viral load, and strain diversity of DWV (Fig. 2). In contrast, neither the prevalence nor the viral load of any of the other four viruses investigated [Kashmir bee virus (KBV), slow paralysis virus (SPV), acute bee paralysis virus (ABPV), or Israeli acute paralysis virus (IAPV)] was affected by the presence of *Varroa* (fig. S1).

In *Varroa*-free areas, DWV was detected in 6 to 13% of colonies, but it increased to 75 to 100% where *Varroa* had been established (Fig. 2). Increased DWV prevalence was accompanied by a millionfold difference in viral load between *Varroa*-free areas (<1000 DWV copies per bee) and *Varroa*-infested areas (>1,000,000,000 DWV copies per bee) (Fig. 2), although there was a time lag associated with changes in strain diversity (that is, between 2009 and 2010 on the Big Island) (Fig. 2 and fig. S2). High-resolution melting (HRM) analysis of DWV–reverse transcription polymerase chain reaction (RT-PCR) products showed that in 2009, 20 colonies from five apiaries, each maintained by independent bee farmers on Oahu, were primarily dominated by a single genotype cluster (Fig. 2 and fig. S2), and sequencing showed that this sequence was identical to DWV sequences previously detected in the United Kingdom, Italy, Denmark, Spain, and France (fig. S3). In 2009, the HRM profiles for Kauai, Maui, and Big Island samples exhibited multiple peaks, indicating the presence of a range of DWV variant sequences (Fig. 2 and fig. S2). Rarefaction analysis of DWV diversity on each island confirmed these findings, with the cumulative number of strains reaching saturation in areas where *Varroa* had been established. In recently invaded or *Varroa*-free regions, the rarefaction curves did not approach saturation, which is typical of highly diverse systems (fig. S4). One year later, *Varroa* had spread across the Big Island, and a follow-up study of 38 colonies from six apiaries showed the same pattern as previously seen on Oahu: an increase in viral load and a decrease in variant diversity (Fig. 2 and figs. S2 and S4). After 1 year of effective *Varroa* control on Oahu, data from 11 colonies in one apiary in 2010 indicated that the same DWV strain remained dominant (fig. S2), suggesting that *Varroa*-induced changes to the viral landscape are capable of persisting despite the *Varroa* populations being under control.

Using 40 clones, sequence analysis revealed 10 virus variants in single bee colonies from each of the four islands, with Kauai, Maui, and the Big Island each having a unique DWV variant (fig. S5). This indicated that a single colony from a *Varroa*-free area contained more viral diversity than that detected across Oahu or the Big Island (in 2010) after *Varroa* had become well established. Subsequent analysis of sequence data separated two distinguishable DWV variant groups (figs. S3 and S5): (i) the "classic" DWV sequence known from symptomatic and asymptomatic honey bees in both *Varroa*-free and *Varroa*-infested colonies; and (ii) a DWV sequence sharing approximately 18 nucleotide substitutions with the closely related *Varroa destructor* virus (VaDV-1) and only 82% sequence homology to the classic DWV sequence (fig. S3).

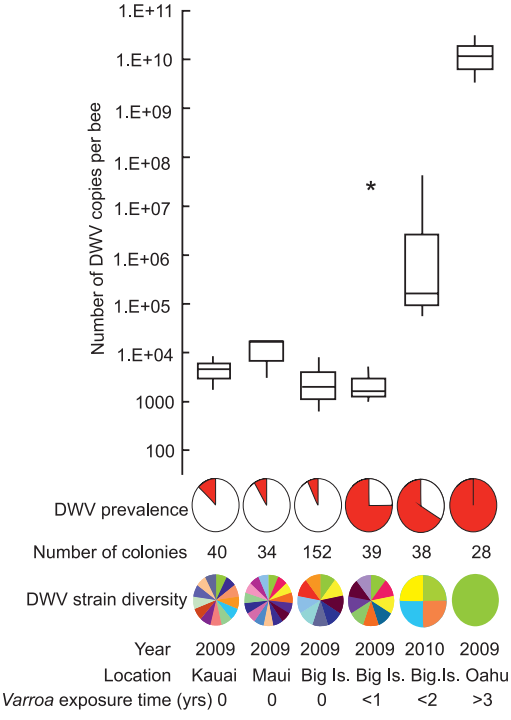

Fig. 2. Viral load, prevalence, and genetic diversity of DWV across the four main Hawaiian Islands that have been exposed to *Varroa* for different periods of time. 1.E + 04 = 1×10^4, etc. The asterisk indicates a *Varroa*-free feral colony that died. Red indicates the proportion of positive colonies (supported by two or more positive RT-PCR tests) in the DWV prevalence pie charts, with the total number of colonies sampled from each population shown beneath. Strain diversity is based on HRM analysis of three randomly selected colonies from each population (figs. S2 and S5) and is supported by the rarefaction curves (fig. S4).

Varroa populations are largely controlled by the use of pesticides, but depending on the season, nearly all bee colonies are infected by DWV (*9*). Such observations are probably due to the fact that *Varroa* is never fully eradicated from infested colonies, and vertical transmission through males (drones) and queens exists (*21*). *Varroa's* arrival at Hawaii has fundamentally altered the viral landscape in both managed and feral bee colonies. On Oahu, all six feral colonies tested had high levels of DWV (6.1×10^8 copies per bee), similar to that found in managed colonies (Fig. 2), whereas only one of nine feral colonies from the *Varroa*-free area of the Big Island carried DWV, and this was the only honey bee colony in a *Varroa*-free area with a high viral level (4.6×10^7 copies per bee) (Fig. 2). This colony had a similar melt curve to that produced by the Oahu cluster and subsequently died within a year. High DWV loads ($>10^7$ copies per bee) have also been associated with colony death in *Varroa*-free areas, indicating that naturally virulent variants can cause colony death, with or without signs of wing deformity, although rarely (*15*).

Variant group A virus is usually associated with symptomatic DWV in the presence of *Varroa*, although it has also been found in *Varroa*-free colonies at significantly lower levels, such as those from Kauai. Rather than resulting from recent recombination events, the B variant and putative DWV/VaDV-1 hybrids (*22*) may simply represent sequence variants that have always existed, and perhaps they highlight the extent of the natural genetic diversity within the collective DWV variants. Furthermore, we found that the Oahu variants had a greater similarity to DWV than to VaDV-1 at regions sequenced on both sides of the proposed recombination points (*22*) (fig. S6). Additionally, leader protein sequence data from Oahu variants clustered with DWV and not VaDV-1 sequences (fig. S7). These findings indicate that the increase in viral load on Oahu is not a result of the formation of DWV/VaDV-1 hybrids. The replicative form of DWV has been detected in mites (*23*), and this study indicates that the presence of *Varroa* over time is selecting for particular variants that may give them a competitive advantage. In Hawaii, the main Oahu strain was also detected in colonies from the Big Island, Maui, and Kauai during 2009 but at much lower frequencies (Fig. 2), supporting the hypothesis that *Varroa* facilitates the dominance of certain strains (*23*), which is strengthened by the loss of strain diversity between 2009 and 2010 as *Varroa* became established on the Big Island (Fig. 2 and fig. S2). Many factors are likely to influence the DWV variant population in different colonies, but the arrival of DWV variants that can replicate in the mite (*13*) means that these strains would rapidly increase in abundance. There have been no major introductions of honey bees into Hawaii, because strict importation regulations have been enacted since the widespread occurrence of *Varroa* mites. It seems likely that the now mite-associated

European DWV variants were already present in honey bee populations before the arrival of the mites. Studies in the United Kingdom (*14*) and New Zealand (*24*) have found that DWV infections and colony collapse did not coincide with the arrival and establishment of *Varroa*, but there was with a 1- to 3-year time lag, which we also observed on Hawaii. This lag appears to be the time required for the selection of virus variants adapted to mite transmission.

Recent studies have found no correlation between the presence of *Varroa* and changes in host immune responses (*10*, *25*, *26*), and the common occurrence of time lags between mite introduction and establishment suggests that the increase in DWV titer and reduction in variant diversity cannot be explained by *Varroa*-induced immunosuppression of honey bees (*27*). The apparent lack of association between ABPV, IAPV, and KBV and *Varroa* in this study may reflect the fact that the latter viruses require a longer lag period to become established in *Varroa* than does DWV, although the prevalence of these viruses varies greatly in *Varroa*-infected areas. Further work is required to elucidate the precise role that *Varroa* may have in influencing the prevalence of the range of viruses that infect bees and their role in colony collapse.

Complete viral genome sequencing and experimental infections of honey bees with different DWV strains are required for testing virulence and *Varroa*-associated honey bee colony losses as was seen on Oahu and the Big Island. The current *Varroa*-adapted DWV variants will continue to evolve, and investigations of virus strain differences may explain the different pathologies currently seen globally in honey bee colonies (*7*). Such variants may interact with other pests, pathogens, environmental factors, and regional beekeeping practices, resulting in recent large-scale losses of honey bee colonies (*6*). This study shows that the spread of *Varroa* in Hawaii has caused DWV, originally an insect virus of low prevalence, to emerge. This association may be responsible for the death of millions of colonies worldwide wherever *Varroa* and DWV co-occur.

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Supplementary Materials

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Vitamin K_2 Is a Mitochondrial Electron Carrier That Rescues Pink1 Deficiency

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Human UBIAD1 localizes to mitochondria and converts vitamin K_1 to vitamin K_2. Vitamin K_2 is best known as a cofactor in blood coagulation, but in bacteria it is a membrane-bound electron carrier. Whether vitamin K_2 exerts a similar carrier function in eukaryotic cells is unknown. We identified *Drosophila* UBIAD1/Heix as a modifier of *pink1*, a gene mutated in Parkinson's disease that affects mitochondrial function. We found that vitamin K_2 was necessary and sufficient to transfer electrons in *Drosophila* mitochondria. *Heix* mutants showed severe mitochondrial defects that were rescued by vitamin K_2, and, similar to ubiquinone, vitamin K_2 transferred electrons in *Drosophila* mitochondria, resulting in more efficient adenosine triphosphate (ATP) production. Thus, mitochondrial dysfunction was rescued by vitamin K_2 that serves as a mitochondrial electron carrier, helping to maintain normal ATP production.

Parkinson's disease (PD) is a common neurodegenerative disorder, and genetic causes of the disease allow us to elucidate the molecular pathways involved (*1*, *2*). Mutations in *pink1*, encoding an evolutionarily conserved mitochondrial kinase, cause PD in humans and mitochondrial defects in model organisms (*3*–*6*). To understand Pink1 function in vivo, we performed a genetic modifier screen in *Drosophila*. Because PD affects the nervous system we screened 193 chemically induced recessive lethal mutants that were selected for defects in neurocommunication (*7*–*9*). We tested dominant modification of $pink1^{B9}$ null mutant flight defects (fig. S1A). Although none of the chemically induced mutants showed dominant flight defects when crossed to a wild-type $pink1^{RV}$ allele, 24 mutants suppressed and 32 enhanced the $pink1^{B9}$ flight defect, such that $pink1^{B9}$ flies failed to fly (fig. S1A).

To reveal the mechanism by which the modifiers affected Pink1, we mapped one of the strongest enhancers that, in combination with $pink1^{B9}$, results in enhanced lethality to *heixuedian* (*heix*). We named this allele $heix^2$ and identified several additional *heix* alleles (fig. S1, B to E) (*10*). To test whether loss of *heix* specifically exacerbated *pink1* phenotypes, we assessed flight, adenosine triphosphate (ATP) levels, and neuronal mitochondrial membrane potential (Ψ_m) (*10*). Heterozygosity for *heix* combined with

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