Title **Deformed Wing Virus is a Recent Global Epidemic in Honeybees driven by Varroa Mites**

Authors: L. Wilfert[1*], G. Long[2], H. C. Leggett[2†], P. Schmid-Hempel[3], R. Butlin[2], S.J.M. Martin[2£]. M. Boots[1$].

Affiliations:

[1]Centre for Ecology and Conservation, University of Exeter, Penryn Campus, Penryn TR11 9FE, UK.

[2]Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK.

[3]Institute of Integrative Biology, ETH Zürich, 8092 Zürich, Switzerland.

*Correspondence to: lena.wilfert@ex.ac.uk

[†]Department of Genetics, University of Cambridge, Cambridge, CB2 3EH, UK.

[£]School of Environment and Life Sciences, University of Salford, Manchester, M5 4WT, UK.

[$]Department of Integrative Biology, University of California, Berkeley, CA, 94720, USA.

Abstract: Deformed Wing Virus (DWV) and its vector *Varroa destructor*, which emerged last century, are a major threat to the world's honeybees. While *Varroa's* dramatic impacts on colony-level DWV epidemiology is evident, we have little understanding of wider DWV epidemiology and the role that *Varroa* has played in its global spread. A phylogeographic analysis shows that DWV is globally distributed in honeybees, having recently spread from a common source, the European honeybee *Apis mellifera*. DWV shows epidemic growth and transmission that is predominantly mediated by European and North American honeybee populations and driven by trade and movement of honeybee colonies. DWV is now an important re-emerging pathogen of honeybees undergoing a worldwide man-made epidemic, fuelled by the novel direct transmission route provided by the *Varroa* mite.

One Sentence Summary: Honeybees are undergoing a DWV pandemic, coinciding with the emergence of the *Varroa* mite, with the global spread driven by Western bee populations.

Main Text: The European honeybee *Apis mellifera* can be argued to be one of the most important domesticated animals, heavily used for commercial pollination of intensive and high-value crops such as the California almond, macadamia, cherries or blueberries as well as honey production. *A. mellifera*, originally from East Asia (*1*), has been intensively managed by beekeepers and exported from its native population in Europe and Africa to the New World and Oceania by European settlers, where beekeeping has become widespread in the last century in line with agricultural intensification. Although wild pollinators play an important role not only for wild flowering plants but also for crop pollination (*2*), our current horticultural systems now heavily rely on managed honeybees, and the global stock of domesticated honeybees is growing more slowly than agricultural demand for pollination (*3*). Understanding the key threats to *A. mellifera* is, as a consequence, clearly important if we are to maintain large populations of bees for both honey production and pollination services. While the number of honeybee hives has increased by 45% on a global scale, there have been dramatic regional declines (e.g. a reduction of 59% in the USA from 1947 to 2005) and beekeepers now globally report high over-wintering colony mortalities, which threaten their sustainability (*4*). While many factors ranging from agricultural intensification to the use of pesticides have been implicated in pollinator declines (*5*), RNA viral infections vectored by the ectoparasitic mite *Varroa destructor* have the potential to be major contributors to global honeybee colony mortalities (*6*). In particular, Deformed Wing Virus (DWV) is the key pathogen associated with over-winter mortality of *Varroa*-infested colonies (*7-10*). The *Varroa* mite jumped from its native host, the Asian honeybee *A. cerana*, to the European honeybee, *A. mellifera*, in the middle of the last century and now has a global distribution (*11*). While DWV occurs in *Varroa*-free natural populations (*12-14*), DWV replicates in the mite (*15*, *16*) or potentially accumulates in its gut ((*17*), but see (*18*)). *Varroa* can inject the virus directly into the bee's hemolymph (*15*, *19*), thus circumventing some of the natural infection barriers to vertical or horizontal transmission between bees, such as the exoskeleton and the peritrophic membranes lining the digestive tract (*20*). Indeed, the recent *Varroa* invasions in Hawaii (*12*) and New Zealand (*13*) led to an increase in DWV prevalence both across colonies and in the viral load in infected individuals, coinciding with a loss in viral diversity. These natural experiments (*12*, *13*) have demonstrated that *Varroa* increases the spread of DWV in honeybee populations. There is also evidence that *Varroa* not only acts as a vector but also increases the virulence of DWV infections, turning relatively asymptomatic infections into ‘overt’ infections associated with clinical disease symptoms (*15*, *21-23*) and increasing winter colony mortalities (*7-10*). There is therefore good evidence that *Varroa* impacts individual and colony-level DWV epidemiology in honeybees, but its importance to the global spread and ongoing worldwide transmission of DWV is unknown. This is an important problem because honeybees today have both a global distribution and a global market. Therefore, we need to understand the factors that drive disease transmission on a global scale in order to be able to limit the spread of the pathogen and mitigate negative effects on beekeeping and the ecosystem services provided by bees (*4*). Furthermore, honeybee diseases also impact the wider pollinator community (*24*, *25*) and we need to understand the global drivers of disease spread to manage disease transfer to novel hosts.

Here, we use a phylogeographic approach to test whether *Varroa*-vectored DWV is a globally emerging honeybee pathogen and to determine the dominant routes of DWV spread. There are two main scenarios for DWV’s origin that can be distinguished based on its phylogeography. The first scenario is that *Varroa* introduced DWV to the European honeybee *A. mellifera* and caused a global epidemic. Under this scenario, we would expect East-Asian *Varroa* populations to be the ancestral host of DWV. The second scenario is that DWV is a re-emerging disease whose current pandemic is promoted by *Varroa*, in which case we would expect *A. mellifera* as the ancestral host. We estimate the major routes of global transmission by comparing geographic and host-specific patterns dated via the viral evolutionary rate, which we have derived for three genomic fragments. A total of 246 DWV sequences were collected from honeybees and *Varroa* mites in thirty-two geographic locations in seventeen countries world-wide, supplemented by all publicly available DWV sequence data, and used to infer the epidemic and migration history driving present-day global DWV dynamics.

From our analysis, DWV shows a recent global radiation and pandemic, with the most recent common ancestor coinciding in time with the global emergence of the *Varroa* mite as a honeybee ectoparasite in the middle of the last century (*11*). The most recent common ancestor for each fragment dates back to the middle of the last century with mean root heights of 44 years (*rdrp*-fragment, 95% Highest Posterior Density (HPD) 27 - 63 years), 47 years (*vp3*-fragment, 95 % HPD 28 – 74 years) and 78 years (*lp*-fragment, 95 % HPD 45 -118 years). All fragments show significant exponential growth over the last decades, with doubling rates around 13 years [*lp*-fragment 16.4 years (95% HPD 9.9 – 46.8 years), *rdrp*-fragment 11.6 years (95% HPD 6 – 96.6 years), *vp3*-fragment 12.4 years (95 % HPD 6.1 – 262.8 years)], which is supported by GMRF skyride analysis (supplementary Fig. S4). Since population structure tends to produce a spurious signature of declining effective population sizes (*26*), we excluded the small number of geographically disparate samples available from Genbank from 2010 for demographic analyses (see Database S1). With the exception of the *rdrp*-fragment, exponential growth is also significant when including samples from 2010-2013. In combination, these results lend support to the hypothesis that DWV has recently radiated from a common source and exponentially spread across the globe (*27*).

While this demographic pattern is consistent with an important temporal role for *Varroa* in the recent expansion of DWV, the global distribution and the ancestral host state of this virus is consistent with DWV being a re-emerging honeybee virus. DWV has been isolated from honeybee populations that had not been exposed to *Varroa* (Australia ((*28*) (HQ655496-HQ655501) and present study, see also Fig. S5), Colonsay Island (Scotland) (14), Hawaii (*12*), Ile d'Oeussant (France) (*14*), Isle of Man (present study), Newfoundland (*29*) and New Zealand (*13*)). This alone would not preclude *Varroa* as the initial source for DWV in *A. mellifera*, as novel emerging pathogens can spread ahead or independently of the initial host if they can replicate in their novel host, as is the case not only in many human zoonoses, such as SARS, but also in wildlife diseases, such as squirrel pox (*30, 31*). Here, *Varroa*, as an active vector that increases DWV prevalence and titer in honeybees (*12, 13*), may increase human-mediated viral spread by increasing the number of infected bees and their transmission potential even without the mite being spread itself. In addition to DWV-presence in *Varroa*-free populations, the phylogenetic reconstruction also contradicts *Varroa* as the ancestral host of the virus. The ancestral host is unanimously identified as *A. mellifera* (state probability P_{lp} = 99.43 %, P_{vp3} = 97.18 %, P_{rdrp} = 92.7 %) – not *V. destructor* (Fig. 1) nor *A. cerana* (Fig. S6 and S7). The geographic origin is less certain with ancestral states being reconstructed with low probabilities, (*lp*-fragment: East Asia, P_{lp} = 69.77 %, *vp3*- and *rdrp*-fragments: Pakistan, P_{vp3} = 77.25%, P_{rdrp} = 54.84%). While we cannot categorically rule out that DWV was introduced to honeybees from an entirely unknown host, this pattern rules out *Varroa* as well as *A. cerana* as the ancestral

DWV-host. The most parsimonious explanation for this pattern is our second scenario: DWV is an endemic honeybee pathogen that has recently re-emerged through ecological change, the spread of *Varroa* as a vector, alongside increased global movement of infected bees or other material such as pollen. This supports previous work postulating that the ancestral form of DWV may have been associated with *A. mellifera* (*32*) and that similarities between DWV lineages may represent a recent introduction from *A. mellifera* into other *Apis* species (*33*).

Our data show that the recent spread of DWV is driven by European *A. mellifera* populations (Fig. 1 & 2a) and shows a similar pattern to the spread of *Varroa* (Fig. 2b), despite increased regulation and control of the global trade in honeybees (*11*). Combining results from the three fragment subsamples for the DWV subtype, Europe, followed by North America, emerge as the main hubs of transmission for DWV to the New World (North and South America and Hawaii) and Oceania (Australia and New Zealand) (Fig. 2 and supplementary table S5). Additionally, there is strong support for migration between East Asia and Europe, with migration being supported in both directions, as well as from Pakistan to Europe in the case of the *vp3*- and *rdrp*-fragments. This pattern overall reflects the invasion pattern of the *Varroa* mite (Fig. 2). Small differences in migration patterns between the fragments may be caused by real biological differences: DWV shows evidence of frequent recombination (*15*) and thus genes may differ in their evolutionary history as well as in their evolutionary rate. However, these differences can also potentially be explained by the different subsets of samples available across fragments (Table S4). Additional analyses to address unequal sample distribution and a sampling bias towards European populations confirmed the predominant pattern of European and North American populations as the main transmission hubs, with some evidence for transmission from Asia to these hubs (Table S6). This analysis also shows strong support for transmission from *A. mellifera* to *V. destructor* for all fragments (Bayes Factor BF$_{lp}$=12281.21, BF$_{vp3}$= 1813.53, BF$_{rdrp}$=12281.21) as well as to other hosts (the common Asian honeybee ectoparasite *Tropilaelaps calreae*, *lp*-fragment BF = 11051.99, and the bumblebee *Bombus lapidarius, rdrp*-fragment BF = 4.62) as shown in Fig. 3. These are not dead-end hosts, with limited evidence for transmission to *A. mellifera* (*V. destructor* to *A. mellifera*: BF$_{lp}$=3.97, BF$_{vp3}$=1813.53, BF$_{rdrp}$=3.09; *rdrp*-fragment: *B. lapidarius* to *A. mellifera* BF=3.74, *Ip*-fragment: T. *clareae* to *A. mellifera* BF=3.93). DWV shows very little host specificity, as the viral population is not structured by host species: $K_{ST,}$ which measures the proportion of genetic variation among populations, is non-significant or close to zero ($K_{ST\_lp} = 0.023$, $K_{ST\_rdrp} = 0.02$, both $p < 0.05$, K_{ST_vp3} n.s.). In contrast, there is significant but overall moderate geographic population differentiation for all fragments ($K_{ST\_lp} = 0.305$, $K_{ST\_vp3} = 0.703$, $K_{ST\_rdrp} = 0.422$, all $p < 0.001$). Population differentiation is significant, but less pronounced within Europe ($K_{ST\_lp} = 0.319$, $K_{ST\_vp3} = 0.135$, $K_{ST\_rdrp} = 0.181$, all $p < 0.001$) and East Asia ($K_{ST\_lp} = 0.301$, $p < 0.001$; other areas/fragments provided too few samples to be informative). Samples that are genetic nearest neighbors largely come from the same population (Hudson's nearest neighbor statistic at continent level: $S_{nn\_lp} = 0.831$, $S_{nn\_vp3} = 0.679$, $S_{nn\_rdrp} = 0.65$, all $p < 0.001$; within Europe: $S_{nn\_lp} = 0.772$, $S_{nn\_vp3} = 0.771$, $S_{nn\_rdrp} = 0.628$, both $p < 0.001$; within East Asia: $S_{nn\_lp} = 0.923$, $p < 0.001$). This indicates that DWV has accrued geographic variation since the origin of the epidemic ~80 years ago, but highlights that high rates of human-mediated migration within Europe and East Asia may obscure population differentiation. It is also evident from the phylogenetic trees (Fig. 1) that *A. mellifera* is the reservoir host for DWV, with other host species clustered at the terminal nodes. Thus DWV apparently has little host specificity, being readily transmitted between different host species, but its primary host is *A. mellifera*, with global transmission having largely been driven by European populations (Fig. 2).

DWV not only causes colony mortality in managed *A. mellifera* populations but also impacts feral populations (*34*) and has been identified as an emerging disease in wild pollinators (*24*, *25*, *35*), with dramatic impacts on survival in bumblebees (*24*). As such DWV may pose a threat not only to managed honeybees but also to pollinators more generally. Wild pollinators such as bumblebees and solitary bees have experienced a loss of species richness and diversity over the last decades, which can partly be attributed to infectious diseases (*4, 36-39*). Our results show that there is a global pandemic of DWV with transmission mediated by European populations of *A. mellifera*. This is an anthropogenic transmission, spread by the global movement of honeybees or other infected material, likely fueled by the concurrent emergence of *V. destructor* mites. This highlights how pollinator populations are globally inter-connected via the trade and movement of managed pollinators, leading to the rapid potential spread of pathogens and parasites across the globe and between species. To control DWV and to reduce the negative effects of DWV on beekeeping and wild pollinators, tighter controls such as mandatory health screening and restricted movement of honeybees across borders should be imposed, with every effort made to maintain the current *Varroa*-free refugia for the conservation of wild and managed pollinators in the absence of this vector.

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Fig. 1: Phylogenetic reconstruction of three fragments of DWV showing host and geographic structure. The figure shows Maximum clade credibility (MCC) trees for the *lp*-fragment (A), *vp3*-fragment (B) and the *rdrp*-fragment (C) of DWV. The branches are colored according to the lineages' inferred geographic origin and the nodes are colored according to the inferred host species. Posterior support >0.5 is indicated for nodes up to the 4th order; horizontal bars indicate the time scale in years. The x-Axis shows time in years. The pie charts show the inferred posterior distribution of the root's geographic location state. See Fig. S3 for an alternative visualization of this graph.

Fig. 2: Global migration patterns of DWV and *V. destructor*. a) Phylogenetically inferred major migration patterns of DWV. The weight of the line indicates the Bayes Factor support for non-zero transition rates (from thin to thick arrows: BF = 3 – 10, 10 – 100, >100) and the color indicates the fragments for which these routes were supported (note that the Thai population was only available for the *lp*-fragment; see Table S5 for detailed results). b) Temporal spread of *V. destructor* in *A. mellifera* based on first records per country (see Materials and Methods); to reflect the coarseness in the data, the temporal spread is indicated by decade. Currently, the only remaining *Varroa*-free large land-masses with a significant honey bee population are Australia and Newfoundland, with mounting evidence that sub-Saharan Africa has been invaded since the turn of the century.

Fig. 3 Phylogenetically inferred DWV-host switching patterns. The weight of the line indicates the Bayes Factor support for non-zero transition rates (from thin to thick arrows: BF = 3 – 10, 10 – 100, >100) and the color indicates the fragments for which these routes were supported

Supplementary Materials:

Materials and Methods

Figures S1-S7

Tables S1-S6

References (*40*-*82*)