HONEYBEE DISEASE

Deformed wing virus is a recent global epidemic in honeybees driven by Varroa mites

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Deformed wing virus (DWV) and its vector, the mite Varroa destructor, are a major threat to the world’s honeybees. Although the impact of Varroa 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 exhibits epidemic growth and transmission that is predominately mediated by European and North American honeybee populations and driven by trade and movement of honeybee colonies. DWV is now an important reemerging pathogen of honeybees, which are undergoing a worldwide manmade epidemic fueled by the direct transmission route that the Varroa mite provides.

The European honeybee Apis mellifera is an important domesticated animal that is used worldwide for commercial pollination of intensive and high-value crops, such as nuts and fruits, as well as for honey production. A. mellifera, originally from East Asia (1), has been intensively managed by beekeepers and exported from its native origins in Europe and Africa to the New World (North and South America and Hawaii) and Oceania (Australia and New Zealand) by European settlers, where beekeeping accompanied agricultural intensification. Although wild pollinators play an important role in the pollination of wild flowering and crop plants (2), our current horticultural systems also rely on managed honeybees. However, the global stock of domesticated honeybees is growing more slowly than the agricultural demand for pollination (3). Understanding the key threats to A. mellifera is important if we are to maintain large populations of bees for honey production and crop pollination services. Although the number of honeybee hives has increased by 45% on a global scale, there have been major regional declines (e.g., a reduction of 59% in the United States from 1947 to 2005), and globally beekeepers have been reporting high overwintering colony mortalities, which threaten the sustainability of bee husbandry (4). Although many factors, ranging from agricultural intensification to the use of pesticides, have been implicated in pollinator declines (5), RNA viral infections transmitted by the ectoparasitic mite Varroa destructor have the potential to be major contributors to global honeybee colony mortalities (6). In particular, deformed wing virus (DVW) is the key pathogen associated with overwinter mortality of Varroa-infested colonies (7–10). The Varroa mite expanded from its native host, the Asian honeybee A. cerana, to the European honeybee, A. mellifera, in the mid-20th century and now has a global distribution (11). Although DWV occurs in Varroa-free natural populations (12–14), DWV appears to amplify in the presence of the mite, either because it can replicate in Varroa (15, 16) or because virus particles accumulate in the mites’ guts (17), but see (18)). Moreover, Varroa can inject the virus directly into the bee’s hemolymph (15, 19), thus circumventing some of the natural barriers to vertical or horizontal transmission between bees, such as the exoskeleton and the peritrophic membranes lining the digestive tract (20). The recent Varroa invasions in Hawaii (22) and New Zealand (13) led to an increase in DWV prevalence among colonies and increased viral loads in infected individuals. Simultaneously, there has been a loss in viral diversity. The Hawaiian and New Zealand invasions (12, 13) indicate that the presence of Varroa increases the spread of DWV across honeybee populations. There is also evidence that Varroa not only acts as a vector but also increases the virulence of DWV infections.

Fig. 1. Phylogenetic reconstruction of three fragments of DWV, showing host and geographic structure. The figure shows maximum clade credibility 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 fourth 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. Fig. S3 provides an alternative visualization of this graph.

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turning relatively asymptomatic infections into “overt” infections associated with clinical disease symptoms (15, 21–23) and increasing colony mortalities in winter (7–10). There is strong evidence that Varroa affects individual and colony-level DWV epidemiology in honeybees, but its importance to the global spread and ongoing worldwide transmission of DWV is less well understood. This is an important problem because of the crucial role that honeybees play in global food production. Furthermore, honeybee diseases also pose risks for the wider pollinator community (24, 25), and we need to understand the global drivers of disease spread in order to manage the transfer of disease to novel hosts.

In this study, we used 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 reemerging disease whose current pandemic is promoted by Varroa, in which case we would expect A. mellifera as the ancestral host. We estimated the major routes of global transmission by comparing geographic and host-specific patterns, dated by the viral evolutionary rate, which we derived for three genomic fragments. We collected a total of 246 DWV sequences from honeybees and Varroa mites in 32 geographic locations in 17 countries worldwide and supplemented these with all known publicly available DWV sequence data; together, these data were used to infer the epidemic and migration history driving present-day global DWV dynamics.

Our analysis shows a recent global radiation and pandemic of DWV, with the most recent common ancestor coinciding in time with the global emergence of the Varroa mite as a honeybee ectoparasite in the mid-20th century (11). The most recent common ancestor for each fragment dates back to the mid-20th century, with mean root heights of 44 years (RdRp fragment; 95% Highest Posterior Density (HPD), 27 to 63 years), 47 years (Vp3 fragment; 95% HPD, 28 to 74 years), and 78 years (Lp fragment; 95% HPD, 45 to 118 years). All fragments show significant exponential growth over past decades, with doubling intervals of ~13 years (Lp fragment, 16.4 years (95% HPD, 9.9 to 46.8 years); RdRp fragment, 11.6 years (95% HPD, 6 to 96.6 years); Vp3 fragment, 12.4 years (95% HPD, 6.1 to 262.8 years)). This finding is supported by a Gaussian Markov random field Skyride analysis (fig. S4). Because population structure tends to produce a spurious signature of declining effective population sizes (26), we excluded the small number of geographically disparate samples available in GenBank from 2010 for our demographic analyses (database S1). With the exception of the RdRp fragment, exponential growth is also significant when including samples from 2010 to 2013. In combination, these results lend support to the hypothesis that DWV radiated recently from a common source and spread exponentially around the globe (27).

This demographic pattern is consistent with Varroa having an important temporal role in the recent expansion of DWV, but the global distribution and the ancestral host state of this virus is also consistent with DWV being a reemerging honeybee virus. DWV has been isolated from honeybee populations that had not been exposed to Varroa [Australia (GenBank accession numbers HQ655496 to HQ655501) (28) and present study; fig. S5]; Colonsay Island, Scotland (14); Hawaii (12); Ile d’Ouessant, France (14); Isle of Man (present study); Newfoundland (29); and New Zealand (13)]. Emerging pathogens can spread ahead or independently of the initial host if they can replicate in newly encountered hosts, as is the case for many human zoonoses such as SARS (severe acute respiratory syndrome) and wildlife diseases such as squirrel pox (30, 31). In this case, because Varroa increases DWV prevalence and titer in honeybees overall (12, 13), it may promote 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 the presence of DWV in Varroa-free populations, the phylogenetic reconstruction also contradicts Varroa
as the ancestral host of the virus. The ancestral host is unambiguously identified as *A. mellifera* (state probability $P_{Lp} = 99.43\%$, $P_{Vp3} = 97.18\%$, $P_{RdRp} = 92.71\%$) and not *V. destructor* (Fig. 1) or *A. cerana* (figs. 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, $P_{Lp} = 77.25\%$, $P_{RdRp} = 54.94\%$). Although we cannot categorically rule out the possibility that DWV was introduced to honeybees from an entirely unknown host, this pattern does rule out *Varroa* and *A. cerana* as the ancestral DWV hosts.

The most parsimonious explanation for the phylogenetic pattern is our second scenario: DWV is an endemic honeybee pathogen that has recently reemerged through ecological change and the spread of *Varroa* as a vector, alongside increased global movement of infected bees or other contaminated material, such as pollen. This conclusion supports previous work that postulated 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 (Figs. 1 and 2A) and follows a similar pattern to the spread of *Varroa* (Fig. 2B), despite increased regulation and control of the global trade in honeybees (7). Combining results from the three fragment subsamples, Europe then North America emerge as the main hubs of DWV transmission to the New World and Oceania (Fig. 2 and table S5). Additionally, there is strong support for migration between East Asia and Europe, in both directions, as well as from Pakistan to Europe in the case of the Vp3 and RdRp fragments. This pattern reflects the invasion pattern of the *Varroa* mite (Fig. 2). Small differences in migration patterns between the fragments may be caused by 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 toward 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 calceatus* ($BF_{Lp} = 11051.99$) and the bumblebee *Bombus lapidarius* ($BF_{RdRp} = 4.621$) (Fig. 3). These are not dead-end hosts, and there is limited evidence for transmission to *A. mellifera* from *V. destructor* ($BF_{Lp} = 3.97$, $BF_{Vp3} = 1813.53$, $BF_{RdRp} = 3.09$), from *B. lapidarius* ($BF_{RdRp} = 3.74$), and from *T. clareae* ($BF_{Lp} = 3.93$). DWV shows very little host specificity, because the viral population is not structured by host species: The $K_{ST}$ statistic, which measures the proportion of genetic variation among populations, is nonsignificant or close to zero ($K_{ST-Lp} = 0.023$, $K_{ST-Vp3} = 0.02$, both $P < 0.05$, $K_{ST-RdRp}$, not significant). In contrast, there is significant but overall moderate geographic population differentiation among all fragments ($K_{ST-Lp} = 0.305$, $K_{ST-Vp3} = 0.703$, $K_{ST-RdRp} = 0.42$; all $P < 0.001$). Population differentiation is significant but less pronounced within Europe ($K_{ST-Lp} = 0.319$, $K_{ST-Vp3} = 0.135$, $K_{ST-RdRp} = 0.38$; all $P < 0.001$) and East Asia ($K_{ST-Lp} = 0.301$, $P < 0.001$; other areas and fragments provided too few samples to be informative). Samples that are genetic nearest neighbors (NN) largely come from the same population, as indicated by Hudson’s NN statistic at the continent level ($S_{NN-Lp} = 0.831$, $S_{NN-Vp3} = 0.679$, $S_{NN-RdRp} = 0.65$; all $P < 0.001$), within Europe ($S_{NN-Lp} = 0.772$, $S_{NN-Vp3} = 0.771$, $S_{NN-RdRp} = 0.628$; all $P < 0.001$), and within East Asia ($S_{NN-Lp} = 0.923$, $P < 0.001$). This result shows that DWV has accrued geographic variation since the origin of the epidemic ~80 years ago, but it indicates that high rates of human-mediated migration within Europe and East Asia obscured population differentiation. The phylogenetic trees (Fig. 1) also show 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 been driven largely by European populations (Fig. 2).

DWV not only causes colony mortality in managed *A. mellifera* populations but also affects 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 during recent decades, which can be attributed partly 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*. Transmission has been amplified by human-mediated movement of honeybees or other infected material and fueled by the concurrent emergence of *V. destructor* mites. Pollinator populations are interconnected via trade and movement of managed pollinators, which offers the potential for rapid spread of pathogens and parasites around the globe and between species. To reduce the negative effects of DWV on beekeeping and wild pollinators, tighter controls, such as mandatory health screenings and regulated 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.

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Fig. 3. Phylogenetically inferred DWV-host switching patterns. The weight of the line indicates the Bayes factor support for nonzero transition rates (as in Fig. 2), and the color indicates the fragments for which these routes were supported. Photo credits are indicated in the figure (CSIRO, Commonwealth Scientific and Industrial Research Organisation).
**FOREST MANAGEMENT**

Europe’s forest management did not mitigate climate warming

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Afforestation and forest management are considered to be key instruments in mitigating climate change. Here we show that since 1750, in spite of considerable afforestation, wood extraction has led to Europe’s forests accumulating a carbon debt of 3.1 petagrams of carbon. We found that afforestation is responsible for an increase of 0.12 watts per square meter in the radiative imbalance at the top of the atmosphere, whereas an increase of 0.12 kelvin in summertime atmospheric boundary layer temperature was mainly caused by species conversion. Thus, two and a half centuries of forest management in Europe have not only failed to contribute to climate change mitigation. The political imperative to mitigate climate change through afforestation and forest management therefore risks failure, unless it is recognized that not all forestry contributes to climate change mitigation.

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during the past few decades, European forests have acted as a carbon sink (1). Forest management, however, can enhance (2) or weaken (3) this sink, which has put it on the political agenda as a mechanism for mitigating climate change (4). However, forest management not only influences the sink strength, it also changes forest structure, which affects the exchange of energy and water vapor with the overlying atmosphere (5–8). Therefore, the potential of forest management to mitigate climate change can only be fully assessed by accounting for the effects from both biogeochemical changes (greenhouse gas emissions) and biophysical changes (water and energy fluxes) (9, 10).

Whereas the effects of historical anthropogenic land-cover changes, such as deforestation and afforestation, on the carbon cycle and the contemporary climate are relatively well documented (11–13), the impacts of land-use changes that do not involve a change in land cover, such as forest management, are far less well understood. Forest management has been reported to affect water and energy fluxes to the atmosphere to the same extent as changes in land cover do (8), suggesting that centuries of forest management may have contributed to Europe’s present-day climate.

Despite the well-known impact of forest management on site-level carbon, energy, and water exchanges (5–8), large-scale studies of the climate effects of forest management were, until recently, hampered by restrictive model approaches and a lack of sufficiently detailed land-use reconstructions. We have addressed both of these limitations. We reconstructed the land-use history of Europe (defined as the land mass west of the Russian border) to take account of both land-cover changes (afforestation and deforestation) and forest management changes (tree species conversion, wood extraction via thinning and harvesting, and litter raking) (14). Among other developments [section 1 of (15)], we then replaced the big-leaf approach in the land-surface model ORCHIDEE-CAN (Organising Carbon and Hydrology In Dynamic Ecosystems—Canopy) with an explicit canopy representation to simulate the biogeochemical and biophysical effects of land-use change (16). The improved land-surface model was coupled to the atmospheric circulation model LMDZ (Laboratoire de Météorologie Dynamique Zoom) [section 2 of (15)] in a factorial simulation experiment to attribute climate change to global anthropogenic greenhouse gas emissions and European land-use change since 1750.

Increased atmospheric greenhouse gas concentrations from global fossil-fuel burning and land-use changes outside Europe are responsible for a change of 2.98 W m⁻² in the radiative imbalance at the top of the atmosphere, a significant increase of 1.71 K in summertime boundary layer temperature ($P < 0.05$, modified $t$ test; Table 1), and an insignificant decrease of 6 mm in summertime precipitation ($P > 0.05$, modified $t$ test; Table 1), relative to 1750. Enhanced plant growth caused by global warming and increased atmospheric CO₂ has resulted in a European forest–based carbon sink of 0.13 Pg C since 1750 (eq. S1), which is a negligible compensation for the contribution of 247 Pg C or 117 ppm CO₂ from global anthropogenic emissions (Table 1).

From 1750 to 1850, deforestation reduced Europe’s forest area by 190,000 km². In the mid-19th century, the increasing use of fossil fuels

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**REFERENCES AND NOTES**

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**SUPPLEMENTARY MATERIALS**

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Materials and Methods

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**REPORTS**

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