Viral transmission in honey bees and native bees, supported by a global black queen cell virus phylogeny

Elizabeth A. Murray[1,2], John Burand[3], Natalia Trikoz[3], Julia Schnabel[3], Heather Grab[1], Bryan N. Danforth[1]

[1] Cornell University, Department of Entomology
[2] current address: Smithsonian National Museum of Natural History, Department of Entomology
[3] University of Massachusetts Amherst, Department of Microbiology

Corresponding author:
Elizabeth Murray
Smithsonian National Museum of Natural History
10th & Constitution NW
Department of Entomology, office CE-509
Washington, DC, 20560

phone: 951-275-3735
email: emurr001@ucr.edu

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Originality-Significance Statement:
Virus transmission across pollinators is an emerging field of research, but nearly nothing is known of virus presence and prevalence in native, solitary bees. We tested for the presence of three different viruses in field-collected managed honey bees and native bees. Our work is one of the few to densely sample two bee groups across several sites and incorporate bee community data to relate those metrics to the incidence of the viruses sampled.

Additionally, we sequenced strains of black queen cell virus and included hundreds of global samples in a dated phylogenetic analysis. This is the first comprehensive, densely-sampled phylogeny of BQCV. A global level phylogeny is essential for deciphering the origin of local viral strains, due to worldwide pathogen movement. Our paper strongly substantiates viral crossover between managed bees and native bees, giving tentative grounds to honey bees as the source. We believe this paper is a valuable contribution to pathogen transmission investigations in bees.

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Summary

In recent decades, we have realized that honey bee viruses are not, in fact, exclusive to honey bees. The potential impact of *Apis*-affiliated viruses on native pollinators is prompting concern. Our research addresses the issue of virus crossover between honey bees and native bees foraging in the same localities. We measured the presence of black queen cell virus (BQCV), deformed wing virus (DWV), and sacbrood virus (SBV) in managed *Apis mellifera* (honey bees) and native *Andrena* spp. (subgenus *Melandrena*) bee populations in five commercial orchards. We identified viral presence across sites and bees and related these data to measures of bee community diversity. All viruses were found in both managed and native bees, and BQCV was the most common virus in each. To establish evidence for viral crossover between taxa, we undertook an additional examination of BQCV where 74 samples were sequenced and placed in a global phylogenic framework of hundreds of BQCV strains. We demonstrate pathogen sharing across managed honey bees and distantly-related wild bees. This phylogenetic analysis contributes to growing evidence for host switching and places local incidence patterns in a worldwide context, revealing multispecies viral transmission.

Introduction

A vigorous supply of honey bees is considered essential to meeting world agricultural needs and providing hive products (Chen and Siede, 2007). This has contributed to ongoing, but recently intensified, widespread public and scientific interest in honey bee health and pathogen management. Honey bees are managed for protection from a variety of bacterial, fungal, viral, and microsporidial diseases, along with parasites, predators, and pests (Vanengelsdorp and Meixner, 2010). Despite this, many honey bee diseases and parasites have a nearly worldwide distribution, reflecting the poor job of

humans thus far in preventing their global spread (Ellis and Munn, 2005; Genersch and Aubert, 2010) and underscoring the deficiencies in our understanding of evolutionary epidemiology (Brown and Fries, 2011).

There is growing realization that many of these honey bee pathogens may not be specific to *Apis* species, though there is little information on virus infections in natural populations of solitary bees – which constitute nearly all bee species (Graystock *et al.*, 2016). It was not until the 2000s that viruses had been characterized in populations of wild bees and other non-managed bee hosts, and it is still most common to measure presence of a virus and not the active infection of the host. Recently this topic has been more actively pursued, and the impact of viruses on the health of native and solitary bees is an emerging field of interest (Singh *et al.*, 2010; Li *et al.*, 2011; Peng *et al.*, 2011; Levitt *et al.*, 2013; Fürst *et al.*, 2014; Ravoet *et al.*, 2014; Graystock *et al.*, 2015; McMahon *et al.*, 2015; Dolezal *et al.*, 2016; Graystock *et al.*, 2016; Tehel *et al.*, 2016; Alvarez *et al.*, 2017; Melathopoulos *et al.*, 2017; Radzeviciute *et al.*, 2017).

Eleven viruses have been found in wild bees, less than half of the number reported from *Apis mellifera* (>26; reviewed in Tehel *et al.*, 2016; Remnant *et al.*, 2017), and data show there is cross-species transmission (Bailey and Gibbs, 1964). Managed bees are found to frequently have a negative effect on wild bees due to their pathogens (Mallinger *et al.*, 2017), and the presence of managed bees can alter pathogen loads in native bees by a number of different mechanisms including facilitation, spillover, and spill back (Graystock *et al.*, 2016). Pathogen transmission dynamics between managed and native bees may also be influenced by the context of the community in which they occur (Power and Mitchell, 2004; Keesing *et al.*, 2006). The diversity of the pollinator community may impact pathogen prevalence by driving niche partitioning or by influencing changes in the activity rate or the level of contact between

infected and susceptible individuals. Knowledge of the role of community composition will be important for understanding the potential impact of pathogen transmission between managed and wild bees, yet to date, we are not aware of studies that have evaluated these relationships in bee communities.

Directionality of virus transmission is difficult to determine; however, a recent study has implicated managed honey bees in transferring viral infections to wild bees (Fürst *et al.*, 2014). It has been established that there is parasite spillover from managed bees which has a negative impact on wild bees (Graystock *et al.*, 2015 and references therein; Graystock *et al.*, 2016), and additionally, data show that prevalence of viruses in honey bees is a predictor of virus prevalence in wild bumble bees (McMahon *et al.*, 2015). In most cases, there is no clear evidence that managed honey bees are the source of the pathogens (Ravoet *et al.*, 2014; McMahon *et al.*, 2015), revealing the insufficiency of information on the interchange of viruses between wild and honey bees.

One important component to determine pathogen origin is the analysis of genetic sequences in a phylogenetic context. By inferring the evolutionary relatedness of pathogens, patterns of transmission can be inferred across time and space, which aids in the understanding of shared strains and, potentially, detection of directionality. Many recent bee virus studies include a phylogenetic component (Singh *et al.*, 2010; Kojima *et al.*, 2011; Zhang *et al.*, 2012; Levitt *et al.*, 2013; Reddy *et al.*, 2013; Yang *et al.*, 2013; Li *et al.*, 2014; Rodríguez *et al.*, 2014; McMahon *et al.*, 2015; Mookhploy *et al.*, 2015; Wilfert *et al.*, 2016; Yañez *et al.*, 2016), though because most studies focused on a limited sample of viruses from a small geographic region, it has not been possible to uncover patterns of inter-continent pathogen transfer (though see Wilfert *et al.*, 2016) and multiple viral introduction events.

This study focuses on three of the most common viruses found in *A. mellifera* in the US (Levitt *et al.*, 2013): black queen cell virus (BQCV) (Dicistroviridae), deformed wing virus (DWV) (Iflaviridae), and sacbrood virus (SBV) (Iflaviridae). These are all positive single-strand RNA viruses in the order Picornavirales. In honey bees, BQCV kills queen bees as pupae, and has been implicated in drone brood death (Siede and Büchler, 2003). While the virus can be detected in honey bee workers, it is not typically a lethal infection in this caste. DWV is often asymptomatic and found at low levels in a honey bee colony, however, direct injection by the *Varroa* mite causes high pathogenicity (Ryabov *et al.*, 2016). This is the most common virus found in non-*Apis* hosts, present in 19 species (Tehel *et al.*, 2016). SBV causes honey bee larvae to die before pupation, and there has been an increased prevalence of this virus in migratory honey bees (Welch *et al.*, 2009). Little is known about the virulence or symptoms of these viruses in solitary bees, and at this time there are few studies that have measured active infection in non-social bees (but see Dolezal *et al.*, 2016).

The potential impacts of *Apis*-derived viruses on native pollinators is a source of concern due to the threat of emerging infectious diseases and the expanding evidence of wild bee population decline. We investigate the patterns of viral presence in managed honey bees (*Apis mellifera*: Apidae) and wild solitary bees (*Andrena*, subgenus *Melandrena*: Andrenidae) in five New York State apple orchards, motivated by evidence that viruses described from honey bees are also carried by wild pollinators. Bees were tested for the presence of BQCV, DWV, and SBV. We were additionally interested to determine if we could ascertain the geographic source of a common pollinator virus and detect ongoing introductions and species crossover. We postulated that extensive sampling would allow us to provide evolutionary context to the viral incidence patterns in a region. Towards this objective, we sequenced BQCV samples from across five sites and placed them in the framework of a large global phylogeny in order to estimate the relatedness and origins of the New York BQCV.

If spillover between honey bees and wild bees is occurring, we predict that the viral presence in *Melandrena* will be positively correlated to both the abundance of honey bees and the viral presence in honey bees, and additionally that a diverse bee community will lower the incidence of infection. We also predict that if spillover is occurring, viral samples from alternate hosts will be interspersed on the phylogeny, indicating that strains are shared between taxa. We found that both wild bees and honey bees may internally carry multiple viruses, though all three viruses tested are more common in honey bees. Our results show strong evidence for virus sharing across various pollinating species and the taxa that inhabit honey bee colonies at not only a regional but also a global scale. These findings indicate that emerging infectious diseases of managed honey bees and native wild bees are intertwined, and evolutionary origins of bee pathogens are best understood in a global context.

Results

Detection of virus presence and virus replication by RT-PCR

In both *Apis mellifera* and native *Melandrena* spp., BQCV was found at all sites and had the highest overall incidence (49.2% of all bees: 71.6% of honey bees and 31.1% of *Melandrena*), followed by DWV (39.0% of honey bees and 10.0% of *Melandrena*) and SBV (26.6% of honey bees and 6.7% of *Melandrena*) (Fig. 1, Table 1). Many bees carried two or more viruses. In *A. mellifera*, 12.8% of individuals tested had all three viruses present, while 0.7% of *Melandrena* were triply infested. Based on a permutation test restricted by site, the number of honey bees with three viruses is higher than expected ($P = 0.000$), and conversely, in *Melandrena* the number is lower than expected ($P = 0.034$) (Fig. S2). For each of the three viruses tested, *A. mellifera* had a higher incidence than *Melandrena* (BQCV: $F_{(1,4)} = 20.937$, $P = 0.0102$; DWV: $F_{(1,4)} = 20.36$, $P = 0.0107$; SBV: $F_{(1,4)} = 15.19$, $P = 0.0175$). The presence of BQCV in both honey bees and *Melandrena* was positively correlated to the presence of DWV ($F_{(1,4)} =$

9.393, P = 0.037) but only honey bees had a marginal positive association between BQVC and SBV (taxa*SBV: z = 1.86, P = 0.062). The presence of DWV in both taxa was positively associated with the presence of SBV ($F_{(1,4)}$ = 9.525, P = 0.0367). The number of significant results explaining viral co-incidence were unlikely to occur by chance (binomial expansion test: i = 2, t = 4, P = 0.014).

Bee community metrics

There was no relationship between presence in honey bees and *Melandrena* for any virus individually (BQCV: $F_{(1,4)}$ = 1.5463, P = 0.281; DWV: $F_{(1,4)}$ = 1.3326, P = 0.312; SBV: $F_{(1,4)}$ = 0.0511, P = 0.832) or for total viral presence (r = -0.503, t = -1.01, P = 0.38). Variation in the composition of the pollinator community across orchards in the year prior to sampling (see Fig. S3) was associated with the incidence of all three viruses. BQCV presence in *Melandrena* was lowest at sites with the greatest Shannon diversity of the bee community (Fig. 2) (z = -4.49, P = <0.001). Honey bee abundance was associated with an increase in DWV presence but only for *Melandrena* spp. (Fig. 3) (z = -2.675, P = 0.007). Oddly, honey bee abundance was also associated with reduced BQCV in *Melandrena* (z = -4.12, P = <0.001). SBV had the lowest incidence in both taxa at sites with high *Melandrena* abundances (Fig. S4) (z = -2.302, P = 0.021) (Table S2, full and simplified models).

Phylogenetic relationships and trait associations

Phylogenetic analyses of BQCV sequences from the current study combined with sequences from GenBank illustrate that BQCV is shared among many different species of hosts globally (Figs 4 and S5 dated phylogeny; Fig. S6 non-dated phylogeny). Hosts collected from 2015 to 1998 (when the first BQCV was sequenced (Leat *et al.*, 2000)) were used to estimate the age of origin of sampled strains at the year 1984 (95% HPD = 1969–1995).

Trait-tip association analysis (Table S4) (Parker *et al.*, 2008) of the 438-taxon BQCV tree reveals that the tips tend to cluster by host (parsimony statistic, $P < 0.0001$); though putatively, collection bias could influence this statistic since there are mainly honey bee hosts in the GenBank database. All categories of hosts besides vespid wasps – *Apis* species, other bee species, bee associates, and the *Ascosphaera* fungus (pathogenic forms cause chalkbrood in honey bees) – were more highly clustered into clades than at random, according to the monophyletic clade statistic. Using geography as a trait, there is also a significant association between geography and phylogeny, and all zoogeographic regions showed higher clustering than random, besides the Neotropical samples, which were not significantly associated with the phylogeny ($P = 1.0$).

Nearly all of the New York bees collected for this study (67 of 74) share closely-related BQCV strains, regardless of host species or collection site (Fig 4B). The remaining seven New York BQCV samples from both *A. mellifera* and *Melandrena* hosts are placed in other locations of the phylogeny (Figs 4, S5, and S6). The fungal *Ascosphaera* samples collected from Maryland honey bees cluster with the majority of the New York samples (0.47 posterior probability (pp)). The estimated age of the New York + Maryland group is dated to the year 2011 (95% highest posterior density = 2012–2011). This clade is most closely related to three honey bee viruses collected in 2006 in Pennsylvania (0.43 pp, Figs 4 and S5).

Trait-tip association analysis of just the 74 BQCV samples from New York indicate there is no pattern of hosts segregating on the phylogeny (Table S4) (parsimony statistic, $P = 0.191$), which is reflected in the monophyletic clade score showing that neither bee group is significantly clustered together (*Apis*: $P = 0.142$, *Melandrena*: $P = 0.089$). However, similar to the global pattern, the New York samples were associated by geographic site (parsimony score, $P = <0.001$). Potentially this was driven by two sites

which had larger clade sizes than the null expectation (site 1: $P = 0.001$ and site 2: $P = 0.001$; sites 3, 4, and 5 each had a non-significant result with $P = 1.000$).

Some caution should be taken in interpretation of the phylogenetic results due to the short length and low variability of the capsid protein 4 marker region used. In the 438-taxon alignment, there are only 158 parsimony-informative characters (22.8%) across the matrix, and 436 characters are identical (62.0%), leading to low differentiation among the samples. Within the 74 New York BQCV samples, 22 characters are parsimony-informative (1.8%).

Discussion

Viral presence

There is convincing evidence from taxonomically-broad collections that honey bee viruses are present in an assortment of arthropods, even beyond the pollinator community (Singh *et al.*, 2010; Levitt *et al.*, 2013). This study shows that the Nearctic *Andrena* (*Melandrena)* not only carry BQCV, DWV, and SBV, but also indicates the active virus replication of BQCV or DWV in 29% of individuals tested.

Multiple virus infections are not unusual in honey bees (Chen *et al.*, 2004 and references therein; Chen *et al.*, 2005). Honey bees are social, which facilitates pathogen transmission between individuals within a colony, and additionally, honey bee colonies are moved around for agricultural purposes, which facilitates interchange across colonies. The greater than expected number of triple positives in our managed honey bees (12.8%) can be explained by the correlation between BQCV presence and the concurrent presence of both DWV and SBV. Potentially this result indicates that foraging honey bees have a higher tolerance to carrying the viruses. The numbers of triply-infested bees are similar to a previous study of BQCV, DWV, and SBV in honey bees in Massachusetts (USA), where it was found that

an average of 11.8% (8.3-18.8%) of the migratory honey bees were triply-positive, however, 0% of the individuals from local honey bee hives were (Welch *et al.*, 2009). Our *A. mellifera* values are much higher than that of a recent survey of honey bee and bumble bee (*Bombus* spp.) virus prevalence across 29 sites in Great Britain, where managed honey bees were triply-positive only in 1% of the cases (McMahon *et al.*, 2015). The wild bumble bees in their study were triply-infested in 0.2% of the cases, which is at a level close to the New York wild solitary bees (0.7%).

We predicted that, should spillover between honey bees and wild bees be occurring, we would recover evidence of shared viral strains. It was verified via phylogenetic methods that BQCV is shared amongst different species of hosts, both in New York and in a wide global sampling of host organisms. Our other prediction of evidence of spillover was that viral presence in *Melandrena* would be positively correlated to honey bee abundance and virus incidence of honey bees. These bee community data are not as straightforward to interpret.

We did not find evidence that viral incidence in honey bees was associated with the incidence in *Melandrena*. However, presence of DWV in *Melandrena* was positively correlated to honey bee abundance in the previous year, and, for *Melandrena*, SBV was negatively correlated to the abundance of *Melandrena* in the previous year at a site (bee community composition was not available from the year of virus sampling). At least for DWV, our data suggests that managed honey bees may increase viral incidence in native bee species.

Currently, there is little data on the mechanisms of virus transmission but there are indications that the foraging process can lead to transmission of disease. High bee abundance may increase contact rates of managed and native bees on shared flowers, which have been implicated as a platform for transfer of

parasites among bee species (Durrer and Schmid-Hempel, 1994; Graystock *et al.*, 2015). It has also been demonstrated that pollen pellets collected from foraging honey bees contain viruses that can be used to infect naïve bee colonies (Singh *et al.*, 2010). The health consequences of the diseased status of solitary bees are virtually unknown, though a recent caged-bee study found that a multi-virus inoculum caused extreme mortality in honey bees but not in two species of solitary bees (Dolezal *et al.*, 2016).

In line with our expectations, bee community diversity was associated with a strong decrease in the presence of BQCV in wild bees, although conversely there were no effects of diversity on BQCV in honey bees or on DWV or SBV. Theory suggests that increasing pollinator community diversity should be associated with a decrease in pathogen prevalence (Keesing *et al.*, 2010), and it is possible that greater diversity may result in a decrease in pathogen prevalence by reducing the abundance of efficient reservoir hosts (Power and Mitchell, 2004; Keesing *et al.*, 2006). The effect of Shannon diversity on BQCV presence in wild bees could putatively be explained in that rented honey bees temporarily introduced to a community may be more insulated to the ameliorating effects of a diverse bee community. Though in cases of high viral incidence (such as our most-commonly sampled BQCV), native *Melandrena* apparently will benefit from higher Shannon diversity.

Our bee community inferences are based on viral presence, so it cannot be ascertained from these data as to how viral infection status would alter the dynamics. Additionally, the consequences of disease in solitary bees is hard to determine since highly infected individuals may not be flying (Fürst *et al.*, 2014). Given the limitations in the spatial sampling of our dataset and lack of information about transmission and virulence it is difficult to draw firm conclusions about the mechanisms of pathogen dynamics. Future studies that quantify viral titer and sample a wider array of species across multiple sites would be able to evaluate the mechanisms proposed here.

Phylogenetic patterns

Worldwide sampling for the phylogeny allows us to investigate both the impact of geography and host on viral evolution, albeit with some caution in interpretation, considering we are reconstructing relationships using a relatively small region of the viral genome. Our BQCV strains can be traced back to 1984, which implies that early history of the virus is not recovered, since BQCV was reported in American honey bees in 1975 (Bailey and Woods, 1977). Still we are confident in the dating and relationships that are encompassed by the phylogeny, which provide strong evidence that in the timespan of the past several years, BQCV has been transferred among multiple host species and across all geographic regions sampled.

Geographic patterns

Despite global honey bee trade, most virus samples tend to group by geography on the phylogeny. We find that viruses from the west Palearctic (Europe) are the sister group to the rest of the BQCV samples. This is a relatively older clade comprised only of honey bee hosts (which may be due to sampling bias). The east Palearctic samples generally occupy a separate phylogenetic area from the majority of the west Palearctic samples, and it was not until 2005 that these BQCV strains entered the east Palearctic (represented by South Korea, China, and Japan). There has been extensive dispersal of the virus in the past decade. No Australian sequences were available, but the dated phylogeny indicates that BQCV had spread worldwide by 2010, when these strains entered the Oriental region (represented by Thailand).

There are a few geographic regions having only one origin on the phylogeny, such as the single group of Kenyan samples (pp = 0.73). However, in regions with repeated sampling over several studies, our large-scale phylogeny reveals potential independent introductions of BQCV to a region. For instance, BQCV

samples are available from five countries in the Neotropics (Brazil 2007, Chile 2010, Costa Rica 2011, Peru 2012, and Uruguay 2005). These are all found in different clades, and none of the countries group together having shared BQCV, indicating these are separate introduction events. Without the broad geographic and taxonomic sampling, this pattern would not have been evident.

In both the worldwide and New York datasets, there was a significant association of geography with phylogeny, indicative that physical location is an important factor in the host-to-host transfer of the virus. Geographic proximity of hosts has been implicated in honey bee to bumble bee BQCV transfer in the US (Peng *et al.*, 2011).

Host patterns

The phylogenetic results indicate non-specificity of BQCV for honey bees. It appears that host transfers occur frequently, as evidenced by the non-*Apis* hosts interspersed across the global BQCV phylogeny. Our regional results across five New York localities show that BQCV is shared between honey bees and wild bees.

Furthermore, using a phylogenetic framework, we show that nest associates can share the same strains as bees. In Japan, a wax moth collected from an *A. cerana* honey bee colony in 2013 has a BQCV strain most closely related to those of adult *A. cerana* from 2012 (pp = 0.68). The Kenyan clade of honey bee adults and brood share related BQCV strains with their *Varroa* mites. The mite *Varroa destructor* vectors several viruses (McMenamin and Genersch, 2015; Ryabov *et al.*, 2016). Additionally, we found that BQCV isolated from the fungus *Ascosphaera apis* from honey bees in Maryland clustered with our New York honey bee and native bee samples, illustrating the close relatedness of the virus and potential for virus crossover between fungal and bee species. Some bee viruses can infect and replicate within

Ascosphaera apis, which causes chalkbrood in honey bees (Li *et al.*, 2014) and is known to be pathogenic in bumble bees (Maxfield-Taylor *et al.*, 2015). Both pathogenic and saprotrophic strains have been found in solitary bees (*Osmia cornifrons*) (Hedtke *et al.*, 2015).

The conclusions we can make about the direction of disease spillover from the worldwide phylogeny are limited by the global sampling bias towards honey bees. In our dated analyses the origin of BQCV is estimated to be older in honey bees than in wild bees. However, no wild bees were sequenced before 2007, which may explain this trend. On a regional scale, BQCV samples from honey bees in Pennsylvania, USA form a grade to the clade of New York and Maryland samples, which tentatively points to honey bees being the origin of infection for the New York bees sampled here.

Implications

We addressed the issue of virus crossover between foraging honey bees and native solitary bees, with the cognizance that the health of honey bees and wild bees is critical for long term, sustainable, crop production (Klein *et al.*, 2007; Blitzer *et al.*, 2016). To infer virus spillover in managed honey bees and wild bee species, we have examined incidence across multiple sites in New York and show evolutionary patterns of BQCV in the state. Our results strongly suggest that New York honey bees and the distantly related ground-nesting bees in the genus *Andrena* subgenus *Melandrena* are sharing viral strains. Furthermore, we are among the first studies to report preliminary evidence of viral replication of both BQCV and DWV in wild caught non-*Apis* bees.

It is now obvious, from our study and others, that many bee and arthropod species carry viruses that have historically been associated only with honey bees; and moreover, we have shown that there is widespread inter-taxon transfer of viral strains. The next step is to determine if the viruses are causing

disease and mortality in solitary bee populations. This is especially critical since we have found high viral presence in the apple-pollinating native *Melandrena*, with half of foraging bees testing positive for at least one of the three viruses and several individuals with signs of active viral replication. Our research indicates that these viruses may have the potential to have widespread effects on wild pollinators.

Experimental Procedures

Background on study system

The bee fauna of New York apple orchards is remarkably diverse, with over 100 wild bee species actively foraging on apple flowers in central New York (Russo *et al.*, 2015). Many commercial growers use managed honey bees for pollination services, yet our estimates suggest that wild bees are actually doing most of the pollination at many sites (Blitzer *et al.*, 2016; Russo *et al.*, 2017). The high species richness of wild bees in New York apple orchards and the heavy use of commercial honey bees at some sites provides an opportunity for substantial pathogen interchange among wild and managed bees.

Field collections

Five field sites were sampled in the state of New York (Fig. 4C). All are commercially managed apple orchards and, in the year of the study (2014), all sites rented honey bee colonies. For viral analyses we collected adult female *Apis mellifera* and *Andrena* subgenus *Melandrena* spp. foraging on apple flowers. *Melandrena* were used because they are some of the most abundant apple pollinators (Russo *et al.*, 2015) and are also easily recognizable to subgenus in the field. *Melandrena* cannot be easily identified to species on the wing, so our samples likely contained a mix of the most common species at each site. Based on historical collections of over 700 specimens collected from 2008 to 2014, we have detected seven species of *Melandrena* at the five focal sites (Fig. S1): *Andrena carlini*, *A. commode*, *A. dunning*, *A.*

nivalis, *A. pruni*, *A. regularis*, and *A. vicina*. Collection frequency data clearly indicates that the two most common species at all our sites are *A. regularis* and *A. vicina*. Only at site 2 is the composition of species markedly different (where *A. pruni* is the most common). Given the close phylogenetic affinities of these species, their similar life histories, and the difficulty of identifying them in the field, we feel justified in lumping samples together as "*Melandrena* spp.".

Detection of virus in samples

Bees were screened for the presence of three viruses: BQCV, DWV, and SBV. A total of 263 bees were collected consisting of 145 *Melandrena* spp. and 118 *Apis mellifera* (Table 1). Of these, 135 *Melandrena* and 109 *A. mellifera* were haphazardly selected to be tested for the three viruses, with 34-47 bees tested per site (Tables 1, S1). Sampled bees were kept chilled and alive, then stored at -80^{o}C. The gut of each bee was dissected from the abdomen and transferred immediately to TRI Reagent (MRC, Inc.) for extraction of RNA per manufacturer's protocol. Gut tissues were crushed with a pestle, centrifuged with chloroform to separate the RNA phase, precipitated and washed with isopropanol and ethanol, and solubilized in water. RNA samples were stored long term in -80˚C for further processing. The RNA samples from bee guts were used as templates in reverse transcription PCR (RT-PCR) with specific primers for the positive strand of DWV, BQCV, and SBV, following previously described protocols (Welch *et al.*, 2009). See the supporting information for primers.

Bee community metrics

We explored the relationship between virus incidence and bee community composition at each site to test whether presence was correlated to the abundance of each host or the diversity of the bee communities at each site using Shannon diversity index for the entire bee community and honey bee or *Melandrena* abundance. Data on abundances and diversity were calculated based on bee community

surveys conducted in the prior year (Park *et al.*, 2015) as temporal auto-correlation analyses indicated that this time lag had the highest correlation (of years tested: 2009-2013), and data for the current year were unavailable (Fig. S3). Community composition data are derived from a long-term dataset of orchard pollinators based on repeated 15-minute transects along orchard rows, in which all bees visiting apple blossoms were netted, preserved and identified to species.

Statistical analyses

Individual bees of *A. mellifera* and *Melandrena* spp. from each field site were scored for presence / absence for each of the three viruses (Table S1). The R package epiR v0.9-93 (Stevenson, 2017) was used to determine 95% confidence intervals around the true incidence of the viruses in each bee group. We set our test sensitivity and specificity to 95%, following previous studies (McMahon *et al.*, 2015; Radzeviciute *et al.*, 2017), and used the recommendation of Reiczigel *et al.* (2010) to use Blaker's method for estimating confidence intervals when there is no prior information on population prevalence. To test if the number of triply-infested bees from each site varied from expected, we developed a permutation test. While maintaining the overall prevalence of each virus within a site to what was observed, outcomes for each of the viruses were randomly reassigned to the individual bees, and the proportion that were triple positives was calculated. This was done 5000 times to generate a null distribution to compare to our observed values.

To test whether the presence of a virus was correlated with the presence of another virus in an individual bee, we used generalized linear mixed models with a binomial error distribution with the R package Lme4 v. 1.1-17 (Bates *et al.*, 2015). Fixed effects included the interaction between taxa (*A. mellifera* or *Melandrena*) and the presence of a second virus. Because viruses covary in presence, only

one virus was used as a predictor in each model, and separate models were fit for each virus combination. Random effects in all models included the nested effects of taxa within site.

We tested the correlation of the rate of incidence of BQCV, DWV, and SBV between *A. mellifera* and *Melandrena* using Pearson's product moment correlation tests. For the bee community composition, binomial generalized linear mixed effects models included fixed effects of taxa, *Melandrena* abundance, *A. mellifera* abundance, and Shannon diversity, as well as the two-way interactions between taxa and each honey bee or *Melandrena* bee community metric. Random effects included species nested within site. Model simplification was performed by backwards-stepwise selection from the full model, and the optimal model was chosen via the best AIC score.

BQCV and DWV sequencing

BQCV positive RT-PCR products were purified with ExoSAP-IT (Affymetrix) and sequenced using primers specific for a ~700 bp region from open reading frame 2 (Leat *et al.*, 2000) of the viral capsid polyprotein (CP4) and the subsequent untranslated region (3'UTR). Chromatograms of BQCV showed that several bees apparently had more than one strain present in otherwise 'clean' strands, as identified as overlapping peaks of two nucleotides on the graph; these were coded as ambiguities. Sequence identity was confirmed by a BLAST search to BQCV sequences on GenBank. Additionally, we sequenced a short protein-coding region of DWV from ten individuals testing positive for DWV presence (see supplementary text for all primers). Annotated sequences have been submitted to GenBank under the accession numbers KY627844-901 & MH931212-27 (BQCV) and MH430173-82 (DWV) (Table S3).

Phylogenetic analyses

In addition to the 74 BQCV sequences obtained from this study (54 from *A. mellifera* and 20 from *Melandrena* spp.), another 366 sequences were included to supplement the taxon sampling and provide a global context (Table S3A). These sequences provide a broad sampling range of both geography and host taxonomy, though there is a strong bias toward viruses obtained from *Apis* spp. in the samples available on GenBank (80.3%). BQCV sequences were aligned using the MAFFT v7 G-INS-i algorithm (Katoh and Standley, 2013): characters 1-546 comprised the capsid protein-coding region 4 (CP4), and 547-703 were non-coding.

Two types of phylogenetic analyses were used for BQCV. First we employed maximum likelihood using the program IQ-TREE 1.6.1 (Nguyen *et al.*, 2015) accessed through the web interface (Trifinopoulos *et al.*, 2016). Following recommended practices, identical sequences were removed before analysis, leaving 352 BQCV sequences. The data was partitioned into coding and non-coding regions, and IQ-TREE automatically chose the best-fit nucleotide substitution models. A consensus tree was calculated.

Second, a dated analyses in BEAST 2.4.6 (Bouckaert *et al.*, 2014) accessed through CIPRES (Miller *et al.*, 2010) incorporated sampling year information for the terminal nodes. In a few instances of no reported date, collection date was estimated to be two years before the sequence was uploaded to GenBank. We used the 438-taxon dataset, removing two questionable sequences from Jordan which were shorter than the rest of the aligned sequences and were on an extremely long branch (Fig. S6, top). The alignment was delimited into three subsets using PartitionFinder1.1.1 (Lanfear *et al.*, 2012) (details in supporting information). For the BEAST dating analysis, the appropriate nucleotide substitution model was applied to each subset, with the gamma parameter for rate heterogeneity estimated. The partitioned clock rates and tree rates were linked, and the data were run under an uncorrelated

lognormal relaxed clock model with a coalescent tree prior with exponential population growth. Three chains were run to >50 million generations and convergence was confirmed in Tracer v1.6 (Rambaut *et al.*, 2013). The runs were combined after removing burnin, and the maximum clade credibility tree was calculated, using median ages.

Additionally, for DWV, we combined our 10 samples from New York with 20 GenBank sequences from the geographic region of the northeast US (Table S3B). The sequence alignment was 483 nucleotides in length, all protein-coding. Using IQ-TREE, we performed a maximum likelihood phylogenetic analysis on these 30 samples (see Fig. S7). A Pennsylvania solitary bee, (*Andrena* spp.), carried a DWV strain identical to our New York viruses.

Phylogenetic trait distributions

Non-random trait association of tips across the phylogeny was tested using BaTS (Bayesian Tip-association Significance testing), beta build 2 (Parker *et al.*, 2008). This program employs statistical measures to test if taxa are more likely than expected by chance to share the same discrete character state. The known trait distribution is compared to a null model of random tip assignments, and statistical significance is calculated. We report the parsimony score, which is the number of state changes on a tree, and the maximum monophyletic clade size. This value is based on the assumption that stronger phylogeny-trait associations will be exemplified by larger clades sharing the same trait at the tips. Only topology is used in the BaTS calculations, and branch length and node support are disregarded. Viruses may be prone to weak phylogenetic signal and high phylogenetic error (Parker *et al.*, 2008), so reconstruction uncertainty is taken into account by testing many trees from the Bayesian posterior distribution. We used 1000 replicates, tested on 1000 trees chosen at random from the post-burnin sample of the dated analysis. Branches below a length of 0.1 were joined into multifurcations.

Host taxon and geography were each used as traits mapped to the dated tree. Host was split into five states: 1) all *Apis* spp., 2) any other bee (solitary or bumble bee), 3) Vespidae: *Vespula* and *Polistes* wasps, 4) *Ascosphaera* fungus, and 5) associates: *Varroa* mites, wax moth, and tenebrionid beetles. Geography was divided into six states: 1) Nearctic, 2) Neotropic, 3) Afrotropic, 4) West Palearctic, 5) East Palearctic, and 6) Oriental (Fig. 4C). Additionally, we tested the New York samples, pruned from the 1000-tree dataset. Host was split into two states: 1) *Apis mellifera*, and 2) *Melandrena* spp. Geography of the New York samples was divided into sites 1-5.

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Tables and Figures

Table 1. Presence of viruses by sample site, for *Melandrena* spp. and *Apis mellifera*.

			BQCV		DWV		SBV	
species	**location**	**# tested**	**# positive**	**%**	**# positive**	**%**	**# positive**	**%**
Melandrena spp.	site 1	28	26	93%	2	7%	**0**	0%
Apis mellifera	site 1	28	19	68%	8	29%	5	18%
Melandrena spp.	site 2	31	4	13%	7	23%	2	6%
Apis mellifera	site 2	24	19	79%	8	33%	5	21%
Melandrena spp.	site 3	28	8	29%	2	7%	2	7%
Apis mellifera	site 3	24	20	83%	8	33%	10	42%
Melandrena spp.	site 4	6	4	67%	**0**	0%	1	17%
Apis mellifera	site 4	28	18	64%	14	50%	9	32%
Melandrena spp.	site 5	42	**0**	0%	2	5%	4	10%
Apis mellifera	site 5	5	2	40%	4	80%	**0**	0%
total *Melandrena*		135	42	31.1%	13	10.0%	9	6.7%
total Apis		109	78	71.6%	42	39.0%	29	26.6%

Figure 1. Viral incidence in New York bees. For *Melandrena*, 136 individuals were tested, and for *Apis mellifera*, 109 were tested. A. Percent viral incidence by bee and virus type. The measured viral presence is shown by a red dash. Bars show the expected true incidence for each virus, with 95% confidence intervals. B. *Melandrena* (55 host individuals total) and, C. *Apis mellifera* (87 individuals) are each shown with the portion of single, dual, and triple virus incidence.

Figure 2. Effect of Shannon diversity of bees on viral presence at a site. Dashed lines represent non-significant associations. BQCV presence in native bees (*Melandrena* spp.) was lowest at sites with the greatest Shannon diversity. Shannon diversity was not associated with presence of DWV and SBV in either taxon (combined in plot) or of BQCV in honey bees. Bee community diversity data is from the year prior to the viral samples, and the 95% confidence interval is shown.

Figure 3. DWV presence as correlated to the average honey bee abundance at a site in the previous year. The x-axis shows the number of honey bees collected by sweep netting a 100 meter transect during a 15 minute period. Shading shows the 95% confidence interval. For DWV, honey bee abundance was associated with a significant increase in DWV presence only for *Melandrena* spp.

Figure 4. A. Maximum clade credibility phylogeny of 438 BQCV virus samples, isolated from: five insect families, the chalkbrood-causing fungus *Ascosphaera* from honey bee hosts, and *Varroa* mites from honey bee colonies. The boxes at the terminals are colored by host category, shown in the legend inset. For readability, we consolidated clades of three or more tips having the same country, host genus, and year, and the number of samples is given in brackets (full phylogeny in Fig. S5). Tips terminate at the

year of the sample collection, given on the bottom axis. Posterior probabilities above 0.1 are shown. B. The clade containing the New York and Maryland samples is shown in the inset tree. C. The five collection localities from this study are shown as numbered dots in the New York State map. All countries represented in the phylogeny are colored on the world map. The branches of the phylogeny are colored according to geographic origin of the sample, as shown on the world map.