

DRIVERS AND PATHWAYS OF VIRUS SPILLOVER WITHIN BEE COMMUNITIES

By

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ABSTRACT

Bees are important pollinators that contribute to both agricultural production and ecosystem function, and stressors including disease, pesticides, habitat loss, and low nutrition negatively impact their health. Honey bee colony losses, which have become increasingly common, have been partially attributed to the *Varroa* mite, which both feeds on the bees and acts as a vector for viruses. Viruses in honey bees have been extensively studied, but there is still much unknown about the risks they pose and how they are transmitted to other bee species, where they have also been detected. The goal of this project was to study virus spillover from honey bees to wild, unmanaged bees to better understand the drivers and transfer pathways of viruses within bee communities. We sampled wild bees, honey bees, and pollen from wild bumble bees from 20 sites varying in agricultural and honey bee densities across Manitoba in the summer of 2024. Bee and pollen samples were individually tested for five common honey bee viruses via RT-qPCR: black queen cell virus (BQCV), sacbrood virus (SBV), deformed wing virus (DWV) A and B, and Israeli acute paralysis virus (IAPV).

We found that virus detections in wild bees are influenced by a variety of factors, and we also found evidence suggesting that virus spillover between honey bees and wild bees is occurring. While wild bees caught at sites with a higher honey bee virus abundance were more likely to harbour viruses, honey bee density was not significant. Other variables associated with virus presence in wild bees, depending on the virus, included agricultural intensity, later collection dates, greater floral abundance, kleptoparasitism, and a closer phylogenetic relation to honey bees. We also found that bumble bees are collecting pollen with viruses present, and these viruses appear to be deposited by other bees present at the same site. Our pollen analysis also indicated that BQCV may be transmitted differently than SBV and DWV-B and may differ in its

ecology. There are a variety of potential mechanisms involved with virus spillover, so to determine the influence of each factor, more experimental transmission and ecological studies are needed.

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The University of Manitoba recognizes that the Treaties signed on these lands are a lifelong, enduring relationship, and we are dedicated to upholding their spirit and intent. We acknowledge the harms and mistakes of the past and the present. With this understanding, we commit to supporting Indigenous excellence through active Reconciliation, meaningful change, and the creation of an environment where everyone can thrive. Our collaboration with Indigenous communities is grounded in respect and reciprocity and this guides how we move forward as an institution.

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INTRODUCTION

Recently reported declines in wild bee populations across the globe have spurred research into the complex causes of declining bee taxa (Goulson *et al.* 2008; Potts *et al.* 2010; Koh *et al.* 2016). Honey bees, a managed species, also experience many of these stressors along with their own set of issues contributing to increased overwintering colony losses (Currie *et al.* 2010). These colony losses have been in part attributed to the ectoparasitic *Varroa destructor*, a mite which feeds on the hemolymph of honey bees and acts as a vector of multiple viruses (Cox-Foster *et al.* 2007; Highfield *et al.* 2009; Currie *et al.* 2010; McMenamin and Genersch 2015). Although these viruses have been best characterized in honey bees, many have also been detected in unmanaged wild bee populations (Fürst *et al.* 2014; McMahon *et al.* 2015; Tehel *et al.* 2016). Little is known about the impact or factors associated with the spillover of these viruses from honey bees to wild bees (Tiritelli *et al.* 2024; Maurer *et al.* 2024), and although shared floral resources are suspected to play a role in virus transfer, there is limited research investigating this transfer pathway (Singh *et al.* 2010; Mazzei *et al.* 2014; Alger *et al.* 2019a, 2019b; Burnham *et al.* 2021).

This thesis is formatted in the traditional style. In this thesis, my first objective is to identify factors associated with virus spillover from honey bees to wild bees. To do this, I tested honey bees and wild bees captured from 20 sites across Southern Manitoba during the summer of 2024. Factors I assessed include agricultural intensity, honey bee density and virus abundance, floral abundance and species richness, day of the year, and wild bee sociality, sex, and phylogenetic relatedness to honey bees. I also constructed phylogenies using virus sequences from PCR products to assess virus sharing between honey bees and wild bees. For my second

objective, I investigate to role of pollen and shared floral resources in virus transfer. I did this by testing pollen samples collected from bumble bees to infer pathways of virus transfer involving shared floral resources between honey bees and wild bees. This work with pollen samples builds upon the research performed by Singh *et al.* (2010), who completed similar research on pollen collected from honey bees.

LITERATURE REVIEW

Wild bee declines

Globally, numerous studies have concluded that wild bee populations are declining (Biesmeijer *et al.* 2006; Goulson *et al.* 2008; Potts *et al.* 2010; Koh *et al.* 2016; Sánchez-Bayo and Wyckhuys 2019; Graham *et al.* 2021; Janousek *et al.* 2023). Wild bees consist of both unmanaged native and exotic species, all of which are interacting with their environment, especially the plants on which they forage (Ayers and Rehan 2023). Wild bee declines are concerning due to our dependence on bees for pollination services and their important role in ecosystems (Klein *et al.* 2007; McMenamin and Flenniken 2018; López-Urbe *et al.* 2020). This is a complex issue, with population dynamics varying across bee taxa, and reductions in bee populations are a result of a combination of factors, many of which are not fully understood (Brown and Paxton 2009; Potts *et al.* 2010; Koh *et al.* 2016; Meeus *et al.* 2018; Mathiasson and Rehan 2020; Graham *et al.* 2021; Janousek *et al.* 2023). Some of the main suspected causes of wild bee declines include pesticide use, invasive species, climate change, and most of all, habitat loss and fragmentation due to agriculture and urbanization (Osborne *et al.* 1991; Biesmeijer *et al.* 2006; Goulson *et al.* 2008; Brown and Paxton 2009; Potts *et al.* 2010; Kerr *et al.* 2015; Koh *et al.* 2016; Woodcock *et al.* 2017; Sánchez-Bayo and Wyckhuys 2019; Mathiasson and Rehan 2020). Another potential driver of these declines is disease, either alone or in combination with other stressors. The role that pathogens play in wild bee declines, whether they are naturally occurring in the population or acquired from managed bee species, is not well understood and poses a considerable knowledge gap (Goulson *et al.* 2008, 2015; Cameron *et al.* 2011; Meeus *et al.* 2018). Some studies have reported on the negative impacts suffered by wild bees in the presence of honey bees, potentially due to interspecific competition (Iwasaki and Hogendoorn

2022). Because these are often correlative studies, it is possible that pathogens spilling over from managed honey bees are in part driving these effects, as many pathogens commonly found in honey bees have also been detected in various wild bee species (Fürst *et al.* 2014; Tehel *et al.* 2016; Iwasaki and Hogendoorn 2022; Tiritelli *et al.* 2024). To better implement conservation strategies for bees, all factors associated with their declines need to be understood, including the impact of diseases.

Bee pathogens

Many pathogens have been found to infect bees, but the pathogens infecting honey bees and other managed bee species, including bumble bees and alfalfa leafcutter bees (*Megachile rotundata* (Fabricius, 1787)), are those that have been best characterized. Some of the main groups of pathogens commonly known to infect bees include bacteria, fungi, mites, and viruses, and the pathogenicity and management of these pathogens is disease dependant. Various factors impact the susceptibility of honey bees to pathogens, including stress, whether that be from other pathogens (Di Prisco *et al.* 2016; Gajda *et al.* 2021), pesticides (Doublet *et al.* 2015; Sánchez-Bayo *et al.* 2016), low nutrition (Dolezal and Toth 2018), or environmental factors (Desai and Currie 2016), and bee genetic factors, including genetic diversity and bees bred for hygienic behaviour (Espregueira Themudo *et al.* 2020; Erez *et al.* 2022). In honey bees, the main bacterial infections that beekeepers manage are American (*Paenibacillus larvae* (White, 1906)) and European (*Melissococcus plutonius* Bailey and Collins, 1983) foulbrood (AFB and EFB), which in Canada can require the destruction of colonies and materials to prevent their spread, specifically in the case of AFB, and the treatment of nearby colonies with antibiotics (Alburaki *et al.* 2024; Micholson 2025). Fungal infections include chalkbrood, which includes species that infect honey bees and managed *M. rotundata* (Vandenberg and Stephen 1982; Aronstein and

Murray 2010), as well as nosema (*Vairimorpha* spp.), a microsporidian genus that is common in honey bees and bumble bees (Otti and Schmid-Hempel 2007; Williams *et al.* 2011). While chalkbrood is not normally managed in honey bee colonies (Aronstein and Murray 2010), the species infecting alfalfa leafcutter bees is the main pest of *M. rotundata*, and as these bees are solitary, their cells are sorted to remove infected individuals before they are placed in fields the following year (Fairey *et al.* 1984). Nosema can be treated in honey bees and involves feeding Fumagilin-B in sugar syrup to infected hives (Williams *et al.* 2011). Mites are a large issue in honey bee management, and include the ectoparasitic *Varroa destructor* Anderson & Trueman, 2000 and *Tropilaelaps* spp., and the tracheal mite *Acarapis woodi* (Rennie, 1921) (Bertola and Mutinelli 2025). *Varroa* mite populations are controlled with acaricide treatments, and *Varroa* monitoring and treatment have been an essential part of maintaining colony health since the emergence of this mite (Rosenkranz *et al.* 2010). Bee viruses, most of which are RNA viruses, are best characterized in honey bees and include many different species, some of which are vectored by *V. destructor* and are therefore controlled through *Varroa* management (Chen and Siede 2007; Locke *et al.* 2012; Grozinger and Flenniken 2019). Multiple viruses found in honey bees are also commonly detected in other bee species, but much less is known about their impact in these groups (Tehel *et al.* 2016).

Pathogen spillover, agriculture, habitat loss, and domestic species

Pathogen spillover, when a pathogen is transferred from an infected population to members of another species, has been best studied in the context of pathogen spillover from wildlife to humans (Otterstatter and Thomson 2008; Williams *et al.* 2021). Many factors increase the risk of pathogen spillover to humans, including habitat loss for the host species, agricultural intensification, and climate change (Saldaña *et al.* 2024). Many of these factors also apply to

pathogen spillover between honey bees and wild bees. Bees are increasingly interacting across landscapes, which could increase the risk of pathogen spillover between bee groups and could negatively impact both managed and wild bee populations. The main interface of these interactions is in the context of agriculture.

Often, agriculture increases the frequency of contact between wildlife and humans, increasing the pathogen spillover risk between these two groups (Williams *et al.* 2021). For example, historical outbreaks of Argentine hemorrhagic fever in humans, an RNA virus transmitted by rodents, overlapped with the timing of corn harvesting when interactions between rodents and workers are elevated (Williams *et al.* 2021). This type of risk posed by agriculture could be similar for bees, as agriculture typically results in monocultures, which could increase overlap in floral resources between honey bees and wild bees in flowering crops while also attracting a high density of bees to a single area. This can increase the risk of pathogen spillover due to increased interactions between bee species that may otherwise forage on different flowering species (Cohen *et al.* 2021; Tiritelli *et al.* 2024; Maurer *et al.* 2024). Agriculture may also impact the health of bees due to pesticide use and low nutrition due to a lack of pollen diversity, or floral resources in many grain crops, increasing their susceptibility to viral infections (Manley *et al.* 2015). Although the impact of agriculture on bee abundance and diversity has been well-studied, the risks associated with agriculture and pathogen transmission are still less clear (Kremen *et al.* 2002; Potts *et al.* 2010; Koh *et al.* 2016).

Related to the risks posed by agriculture are those posed by habitat loss. Encroachment into habitats of disease vectors through urban and agricultural development has increased transmission risk to humans and mammalian livestock, resulting in novel zoonotic outbreaks (Chua *et al.* 2002; Karesh *et al.* 2012; Faust *et al.* 2018; Williams *et al.* 2021). For example, in

Malaysia the 1998 outbreak of Nipah virus in domestic pigs was attributed to deforestation burns, which caused fruit bats carrying the virus to roost in pig barns, subsequently infecting the pigs which then infected humans (Chua *et al.* 2002; Pulliam *et al.* 2012; Williams *et al.* 2021). As in the case of bats and Nipah virus, much of the habitat loss for wild bees has been due to land-use change for agriculture (Brown and Paxton 2009; Potts *et al.* 2010; Kline and Joshi 2020). Although this does not result in bees entering urban areas and spreading pathogens to humans or mammalian livestock, wild bees as a result are present in these agricultural landscapes, which were once their natural habitats, where they now interact with domesticated honey bees. Additionally, bees can act as vectors of plant pathogens in these agricultural landscapes (McArt *et al.* 2014; Pattemore *et al.* 2014).

Most studies focus on the risks associated with pathogen spillover from wildlife to humans or domestic animals, or from domestic animals to humans (Karesh *et al.* 2012; Wells *et al.* 2020; Williams *et al.* 2021; Plowright *et al.* 2024; Saldaña *et al.* 2024). The main difference between these cases and pathogen spillover in bees is that wild bees appear to be the recipients of pathogen spillover more often than honey bees, the domesticated species, although the potential for pathogen spillback to honey bees should still be considered (Tehel *et al.* 2016, 2022; Piché-Mongeon and Guzman-Novoa 2024; Maurer *et al.* 2024). One study using a large global database found that domesticated species carried a greater proportion of zoonotic viruses when compared to wildlife (Wells *et al.* 2020). Domestic species in particular may play a greater role in virus transmission both because of their large geographic range, and the high densities and large populations in which they live (Wells *et al.* 2020). Unlike many domestic animals which are kept in enclosures or fenced pastures, honey bees freely interact with their surroundings and

cannot be confined to a specific area. This makes honey bee-wild bee pathogen spillover a somewhat unique and interesting case to study.

Honey bees and wild bees

The western honey bee, *Apis mellifera* L., is the most widely known bee species, as it is managed for both honey production and crop pollination across all continents except Antarctica (Moritz *et al.* 2005). Both managed honey bees and wild, unmanaged bees experience similar stressors including pathogens, pesticides, low nutrition, and habitat loss, with honey bees being the most thoroughly studied bee species (Potts *et al.* 2010; Goulson *et al.* 2015; McMenamin and Flenniken 2018; Klein *et al.* 2018; López-Urbe *et al.* 2020). Honey bees are not the only important pollinating bee; wild bees, most of which are native to their environment, provide important pollination services and have been shown to be more effective pollinators of certain crops including cherries (Bosch and Kemp 1999), strawberries (MacInnis and Forrest 2019, 2020), and certain apple cultivars (Vicens and Bosch 2000). This pattern held true in a worldwide study assessing 41 crops, in which wild pollinators had a greater positive effect on fruit set (Garibaldi *et al.* 2013). In areas where honey bees are introduced, pollination provided by honey bees is likely least important in non-agricultural ecosystems, where native bees already effectively provide pollination services, and most valuable in agricultural landscapes and disturbed ecosystems with reduced pollinator abundance (Dick 2001; Aizen and Harder 2009; Reilly *et al.* 2020; Page and Williams 2023).

While honey bees (*A. mellifera*) are eusocial insects that live in colonies within a cavity, wild bees are a diverse group with many different social structures, nesting strategies, and foraging patterns (Gibbs *et al.* 2023). Depending on various functional traits, pathogen spillover risk may be enhanced or lessened in certain groups (Figueroa *et al.* 2021; Tiritelli *et al.* 2024).

While some wild bees are generalist pollinators (polylectic) like honey bees, others specialize on specific flowering species or taxa (oligolectic) (Gibbs *et al.* 2023). Generalist bees foraging on a broader range of flowering species may be more likely to forage on the same flowers as honey bees compared to a specialist bee, and therefore may be at a greater risk of pathogen spillover (Maurer *et al.* 2024). Conversely, foraging on many flowering species may dilute the risk of pathogen transfer in polylectic bees when compared to oligolectic bees, if the specialist bee forages on a flowering species that many bees may visit (Tiritelli *et al.* 2024). Additionally, nest type could impact pathogen risk, as bees that reuse cavities for nests previously used by other bees could be at risk of pathogens left by the previous occupants, or bees nesting below ground could live in an environment that promotes pathogen persistence through low temperatures and a lack of sunlight (Tiritelli *et al.* 2024). Bivoltine bees with multiple generations in a year could also be at greater risk of certain vertically transmitted pathogens if infections are amplified between generations (Tiritelli *et al.* 2024). While western honey bees are a single species with known pathogen transmission pathways, wild bees exhibit diverse traits across species that could influence patterns of virus risk and spillover.

Honey bee colony losses and viruses

Wild bee contributions to pollination may become even more important as honey bee overwintering colony losses increase (Kremen *et al.* 2002; Winfree *et al.* 2007; Currie *et al.* 2010), which in 2025 were 39.3% in Canada (Claing *et al.* 2025). These colony losses, in conjunction with other stressors, have been attributed to both the ectoparasitic mite *Varroa destructor* and the viruses it acts as a vector for, primarily deformed wing virus (DWV) and Israeli acute paralysis virus (IAPV) (Cox-Foster *et al.* 2007; Highfield *et al.* 2009; Currie *et al.* 2010; de Miranda and Genersch 2010; McMenamin and Genersch 2015). *Varroa destructor*

feeds on hemolymph and is dependent on honey bees during all life stages, feeding and reproducing within brood cells and feeding during its phoretic stage, in which it can move between hosts (Chen and Siede 2007; Rosenkranz *et al.* 2010). The mite alone causes many harmful symptoms including reduced bee weight and lifespan, reduced immune function, and eventual colony collapse (Chen and Siede 2007; Rosenkranz *et al.* 2010). DWV, which includes both DWV-A and the potentially more virulent variant DWV-B (formerly VDV-1: *Varroa destructor* virus-1), is commonly associated with high mite loads in colonies and colony losses, has been shown to replicate within *Varroa* mites, and causes symptoms including deformed wings and shortened lifespans in adult honey bees (Gisder *et al.* 2009; de Miranda and Genersch 2010; McMenamin and Genersch 2015; McMahon *et al.* 2016; Martin and Brettell 2019; Paxton *et al.* 2022). IAPV is also associated with colony losses and *Varroa* mites, and symptoms include bee paralysis and mortality (Cox-Foster *et al.* 2007; Di Prisco *et al.* 2011; McMenamin and Genersch 2015; Deng *et al.* 2021).

Along with DWV and IAPV, other honey bee viruses common in Manitoba include black queen cell virus (BQCV) and Sacbrood virus (SBV) (Chen and Siede 2007; Desai *et al.* 2016; Robson-Hyska 2017; Sharma 2025). BQCV is primarily symptomatic in developing honey bee queens, causing infected larvae to turn black and die, followed by the cell turning similarly dark; hence the virus' name (Bailey and Woods 1977; Chen and Siede 2007). Similar symptoms have infrequently been reported in drone brood (Siede and Büchler 2003). Workers infected with BQCV alone are generally not known to be symptomatic, although there is some evidence that their orientation may be affected by infections (Chen and Siede 2007; Retschnig *et al.* 2019). Sacbrood virus also primarily affects developing bee larvae, although it also can affect worker bees (Anderson and Gibbs 1989; Chen and Siede 2007). The name of the virus is descriptive of

the symptoms observed in infected larvae, which are leathery skin and fluid buildup under the skin prior to larval death (Bailey *et al.* 1964; Chen and Siede 2007). Symptoms in infected adults are less evident, but include shortened lifespans and reduced pollen foraging (Bailey and Fernando 1972; Anderson and Giacon 1992a).

While DWV and IAPV are highly associated with *V. destructor*, the associations between *Varroa* mites and BQCV, and *Varroa* and SBV are less clear (Gisder *et al.* 2009; Di Prisco *et al.* 2011; McMenamin and Genersch 2015). Both viruses have been detected in *Varroa* mites, so the mite may act as a vector, but a study by Locke *et al.* (2012) showed that while a significant positive relationship was observed between *Varroa* mite infestations and DWV, there was not a significant relationship between mite infestations and BQCV or SBV (Shen *et al.* 2005; Chen and Siede 2007). Conversely, BQCV is known to be associated with nosema, which infects the honey bee midgut epithelium and for some species (*Vairimorpha apis* (Zander, 1909)) causes dysentery, potentially due to nosema infections allowing BQCV to more easily infect bees using the weakened gut as a route of entry (Bailey *et al.* 1983; Berényi *et al.* 2006; Chen and Siede 2007; Galajda *et al.* 2021; Tiritelli *et al.* 2024). Honey bees with a coinfection of nosema and BQCV have been demonstrated to have a greater mortality than those infected by nosema alone, whether it be by *V. apis* or the more virulent *V. ceranae* (Fries, Feng, da Silva, Slemenda & Pieniazek, 1996) (Bailey *et al.* 1983; Gajda *et al.* 2021).

Along with transmission via *Varroa* mites, honey bee viruses have been demonstrated to be transmitted through a variety of other intraspecific routes. Vertical transmission of viruses, transmission from the queen to her offspring, has been detected in numerous studies, where the eggs laid by queens have detectable virus that would not be acquired through other horizontal routes (Chen *et al.* 2006a, 2006b; Chen and Siede 2007; de Miranda and Fries 2008; Ravoet *et*

al. 2015; Amiri *et al.* 2018). Venereal transmission is also possible, where viruses are passed from drones to a queen during mating, and has been best characterized in DWV (Yue *et al.* 2007; Chen and Siede 2007; de Miranda and Fries 2008; Amiri *et al.* 2016; Yañez *et al.* 2020). Another horizontal transmission route is foodborne transmission, which can occur through trophallaxis during the processing, storage, and feeding of food to other bees. Because nectar is stored internally while bees forage, then regurgitated and further processed by nurse bees, and because royal jelly is a secretion made by bees, this provides a pathway for virus transmission (Yue and Genersch 2005; Chen *et al.* 2006a, 2006b; Chen and Siede 2007). Fecal-oral transmission is closely related to foodborne transmission, where feces containing viral particles are consumed by another individual, leading to infection. This often occurs in hives where many individuals live in close proximity and hive surfaces, including food, become contaminated by feces, but it could also occur outside the hive (Chen *et al.* 2006a, 2006b; Chen and Siede 2007; Singh *et al.* 2010; Koch *et al.* 2017; Grozinger and Flenniken 2019; Yañez *et al.* 2020). Viruses are likely transmitted between bees through the sharing of floral resources, combining fecal-oral and foodborne transmission routes, as discussed in a later section (Singh *et al.* 2010; Koch *et al.* 2017; Alger *et al.* 2019b; Dalmon *et al.* 2021; Burnham *et al.* 2021).

These four honey bee viruses are all found within the order Picornavirales, making them positive-sense RNA viruses; DWV and SBV belonging to the family Iflaviridae, and IAPV and BQCV belonging to the family Dicistroviridae (de Miranda *et al.* 2013; Kwon *et al.* 2023). The RNA viruses discussed here all harbour a high mutation rate due to their lack of proofreading mechanisms during replication (Bonhoeffer and Sniegowski 2002; Chen and Siede 2007; Wells *et al.* 2020; Williams *et al.* 2021). This plasticity allows them to quickly adapt, evolve, and potentially exploit new related hosts due to their high genetic diversity (Bonhoeffer and

Sniegowski 2002; Chen and Siede 2007; Wells *et al.* 2020). It has been found that hosts of the same RNA virus are more likely to be phylogenetically closely related species, and although this research is mostly restricted to research in mammals, it likely applies to other animal taxa, including bees (Wells *et al.* 2020). Because of their high mutation rate, each of the above viruses is more accurately described as a “quasispecies” with high levels of genetic variation, including variation within each individual infection (Domingo 1997; Bonhoeffer and Sniegowski 2002; Chen and Siede 2007; Luring and Andino 2010). Many phylogenetic trees have been constructed to elucidate the phylogeny of numerous viral variants detected in honey bees and other bee species (Singh *et al.* 2010; Levitt *et al.* 2013; Fürst *et al.* 2014; Murray *et al.* 2019; Dalmon *et al.* 2021).

Honey bee viruses across species

Although many studies have examined the impact of honey bee viruses on honey bees, fewer have focused on the impact of these viruses on wild bees (Fürst *et al.* 2014; Tehel *et al.* 2016; Dolezal *et al.* 2016). Most work to date has focused on detecting viruses in various bee species, with a focus on DWV and BQCV in *Bombus* spp. (Fürst *et al.* 2014; Tehel *et al.* 2016; Radzevičiūtė *et al.* 2017; Martin and Brettell 2019; Alger *et al.* 2019a; Tapia-González *et al.* 2019). In addition to Apidae, honey bee viruses have been detected within the bee families of Megachilidae, Halictidae, Andrenidae, and Colletidae (Tehel *et al.* 2016; Dolezal *et al.* 2016; Radzevičiūtė *et al.* 2017; Tapia-González *et al.* 2019; Dalmon *et al.* 2021; Nanetti *et al.* 2021; Deutsch *et al.* 2023). A study by Fürst *et al.* (2014) in Great Britain and the Isle of Man found that there was a significant positive effect of DWV detections in honey bees on DWV detections in *Bombus* spp. across 26 sites. An associated study published the following year using these same sites detected a positive relationship between BQCV prevalence in bumble bees and both

BQCV prevalence in honey bees and honey bee abundance (McMahon *et al.* 2015). Similar results were reported by Alger *et al.* (2019a), who found DWV and BQCV infections in *Bombus* spp. were greater in the presence of apiaries. Piot *et al.* (2022) also found that the viral prevalence of three viruses in honey bees and wild bees were positively related, and Maurer *et al.* (2024) found a similar positive association between BQCV loads in honey bees and wild pollinators. Some studies have also sequenced honey bee viruses found in honey bees and wild bees, and it is generally found that within phylogenetic trees, the viruses are not clustered based on host species, and if they are clustered, it is based on location, supporting the hypothesis that these viruses are shared among bee species (Singh *et al.* 2010; Levitt *et al.* 2013; Fürst *et al.* 2014; Radzevičiūtė *et al.* 2017; Murray *et al.* 2019; Dalmon *et al.* 2021).

Because most common honey bee viruses are positive-sense RNA viruses, their genome is normally just a single positive-sense strand of RNA (positive-strand), but during viral replication, a transient negative-sense strand is produced (negative-strand) (Chen and Siede 2007; Warncke and Knudsen 2022; Kwon *et al.* 2023). This replicative strand can be specifically detected using modifications to the typical PCR virus detection protocol (Warncke and Knudsen 2022). Some studies have detected the negative-strand of some honey bee viruses within other bee species, indicating that some viruses can replicate within certain wild bee species (Fürst *et al.* 2014; Radzevičiūtė *et al.* 2017; Tapia-González *et al.* 2019; Morfin *et al.* 2021b). These include many *Bombus* spp., other species within Apidae, and species within Andrenidae, Megachilidae, Halictidae, and Colletidae (Mazzei *et al.* 2014; Radzevičiūtė *et al.* 2017; Tapia-González *et al.* 2019; Fearon and Tibbetts 2021; Morfin *et al.* 2021b; Schauer *et al.* 2023).

Some *Bombus* species infected with DWV have been shown to exhibit symptoms including deformed wings and increased mortality (Genersch *et al.* 2006; Fürst *et al.* 2014;

Graystock *et al.* 2016b; Streicher *et al.* 2023). IAPV has also been shown to cause symptoms of decreased reproduction and increased mortality in *Bombus terrestris* (Linnaeus, 1758) when individuals are infected through oral inoculation (Meeus *et al.* 2014a; Piot *et al.* 2015). Outside of *Bombus*, survival was shown to be reduced in the stingless bee *Melipona beecheii* Bennett, 1831 when fed either DWV-A, DWV-B, or BQCV (Fleites-Ayil *et al.* 2023). Another study found that after being fed a mixture of BQCV, DVW, IAPV, and SBV, in a dose tested to be lethal to honey bees, *M. rotundata* and *Colletes inaequalis* Say, 1837 had virus titres that either decreased or did not change significantly over the course of five days following inoculation, suggesting that these two species may not be particularly susceptible to these viruses (Dolezal *et al.* 2016). Notably, this mixture contained 89% SBV, a virus best known for its symptoms in young larvae, while symptoms in adult honey bees can be less obvious (Bailey and Fernando 1972; Anderson and Giacon 1992b; Chen and Siede 2007). So, although this dose of virus mixture was lethal to honey bees, testing its effects on *M. rotundata* and *C. inaequalis* larvae may yield different results, and testing the effects of individual viruses separately may be more informative. Still, these findings were partially supported by those of Melathopoulos *et al.* (2017), which reported little evidence of virus transfer from honey bees to *M. rotundata* in their field study, although they did find that SBV may impact cocoon viability which was reduced at sites with SBV present in adults. Again in *M. rotundata*, Singh (2011) found that when fed IAPV, newly emerged bees had shortened lifespans, and larvae were more likely to emerge as second-generation adults rather than enter diapause when compared to the control larvae. In an inoculation experiment studying *Osmia bicornis* (Linnaeus, 1758), adults injected with DWV-A showed decreasing virus titres over the course of the experiment, and inoculated bees did not show increased mortality compared to controls, although low levels of replication were detected

(Schauer *et al.* 2023). There appears to be variation in susceptibility between different bee species to each virus, as indicated by the results of McMahon *et al.* (2015) in relation to acute bee paralysis virus (ABPV) and slow bee paralysis virus (SBPV) prevalences across bumble bee species. More research is needed to better understand differences in virus susceptibility, and to identify which species are most at risk from each honey bee virus.

Honey bee viruses have also been detected outside of bees (Anthophila), and even outside of insects (Insecta). These detections include the hymenopteran families of Formicidae, Vespidae, and Scoliidae, and the orders Blattodea, Dermaptera, Coleoptera, Diptera, and even Araneae (Celle *et al.* 2008; Levitt *et al.* 2013; Forzan *et al.* 2017; Nanetti *et al.* 2021; Power *et al.* 2023; Dobelmann *et al.* 2023; Baril *et al.* 2023; Schläppi *et al.* 2023; Deutsch *et al.* 2023; Eroglu 2023; Mehmood *et al.* 2023; Maurer *et al.* 2024). Viral symptoms have also been observed within *Vespa* spp. (Hymenoptera: Vespidae) and *Agelena labyrinthica* (Clerck, 1757) (Araneae: Agelenidae), both of which feed on honey bees (Forzan *et al.* 2017; Schläppi *et al.* 2023; Mehmood *et al.* 2023). *Vespa velutina* Lepeletier, 1836 and *Vespa crabro* Linnaeus, 1758 have been observed with deformed wings when infected with DWV-A, and an individual *V. velutina* exhibited decreased hair and colour when infected with IAPV and DWV-A (Forzan *et al.* 2017; Mehmood *et al.* 2023). Based on experiments with *A. labyrinthica*, DWV-B appears to reduce cocoon production in the species (Schläppi *et al.* 2023). Because some honey bee viruses have been shown to induce adverse symptoms in species so distantly related to honey bees, it seems likely that these viruses could be negatively impacting a greater number of bee species than have been reported so far.

Insect immune systems differ greatly from those of vertebrates, which include both innate and adaptive responses, whereas insects have only innate, nonspecific immune responses (Chen

and Siede 2007). Still, insects have multiple immune pathways in place against pathogens, and although these have been best characterized in the model organism *Drosophila melanogaster* Meigen, 1830 and important disease vectors *Aedes aegypti* (Linnaeus, 1762) and *Anopheles gambiae* Giles, 1902, honey bees have also been the focus of multiple studies and reviews (Evans *et al.* 2006; Waterhouse *et al.* 2007; Brutscher *et al.* 2015, 2016; McMenamin *et al.* 2018; Morfin *et al.* 2021a). Immune responses are carried out through multiple mechanisms, and those best characterized in honey bees include RNA interference (RNAi), the Toll, Jak/STAT, Imd, and JNK signal transduction pathways, and mechanisms including phagocytosis, nodulation, and melanization (Evans *et al.* 2006; Brutscher *et al.* 2015; McMenamin *et al.* 2018; Morfin *et al.* 2021a). In antiviral responses, RNAi appears to be an important mechanism where dsRNA (double-stranded RNA) is cleaved by Dicer, an RNase endonuclease, which produces small interfering RNAs (siRNA) (Chen and Siede 2007; Brutscher *et al.* 2015, 2016; McMenamin *et al.* 2018; Morfin *et al.* 2021a). This siRNA then integrates with an RNA-induced silencing complex (RISC), activating the complex and acting as a template to degrade other RNA molecules with homologous sequences (Chen and Siede 2007; Brutscher *et al.* 2015, 2016; McMenamin *et al.* 2018; Morfin *et al.* 2021a). Some signal transduction pathways, which result in the synthesis of AMPs (antimicrobial peptides) have also been implicated in antiviral responses, but the viral defense mechanisms are less well characterized compared to RNAi (Brutscher *et al.* 2015; McMenamin *et al.* 2018; Morfin *et al.* 2021a).

Virus transmission via pollen

Fecal deposits on flowers and their pollen are thought to be one of the main modes of virus transmission from honey bees to wild bees since they share floral resources, but research on this topic is limited (Singh *et al.* 2010; McArt *et al.* 2014; Koch *et al.* 2017; Pereira *et al.* 2019;

Alger *et al.* 2019a, 2019b; Schittny *et al.* 2020; Burnham *et al.* 2021; Balkanska *et al.* 2023).

Burnham *et al.* (2021) found that foraging on red clover flowers (*Trifolium pratense* L.) in cages could successfully transfer DWV to virus-free bumble bees (*Bombus impatiens* Cresson, 1863) when flowers were either inoculated by hand with field-realistic doses of DWV or inoculated by placing the flowers in cages with infected honey bees. In addition, they found that DWV infected bumble bees could deposit virus on artificial flowers when foraging (Burnham *et al.* 2021).

Honey bees were also shown to deposit viruses on flowers in a study by Alger *et al.* (2019b), although this study didn't detect viral transmission to bumble bees that foraged on the flowers following virus deposition. A study by Tehel *et al.* (2022) demonstrated that when bumble bees (*B. terrestris*) or honey bees were fed from feeding tubes previously used by infected honey bees, DWV-A transmission was possible, but transmission from infected bumble bees to honey bees or between bumble bees in the same setup was not detected. Additionally, when housed in the same cage, honey bees readily transmitted DWV-A to bumble bees and other honey bees, although infected bumble bees did not transmit DWV-A to honey bees or other bumble bees (Tehel *et al.* 2022). This could mean that while honey bees can transmit viruses to other bees, other bee species may not be spreading viruses following infection, although this may be virus-dependant. Lending more evidence to virus spillover through floral resources, a field study found that 19% of flowers near apiaries had detectable DWV or BQCV, whereas no flowers collected in the absence of apiaries and honey bees had detectable virus (Alger *et al.* 2019a). Additionally, flowers and pollen have been implicated in the transmission of other pathogens between bees (McArt *et al.* 2014; Graystock *et al.* 2015; Koch *et al.* 2017; Pereira *et al.* 2019; Pinilla-Gallego *et al.* 2022).

It was previously thought that viruses were transferred by corbiculate bees to pollen through infected saliva used to help pollen clump during foraging (Singh *et al.* 2010). A study by Singh *et al.* (2010) found that the viruses detected in pollen pellets do not necessarily reflect those detected in the honey bee forager carrying them, and virus sequences from a forager and her pollen often do not match. This indicates that viruses may be spread by fecal deposits from bees on flowers, or another pathway involving bee traffic on flowers, rather than through the salivary glands of the bees collecting the pollen (Singh *et al.* 2010; Colwell *et al.* 2025). This seemed to be virus-dependant, so while there was a positive correlation between forager and pollen pellet DWV prevalence, this was not the case for SBV or BQCV (Singh *et al.* 2010). This was demonstrated only in honey bees in this study; only three wild bees were tested for viruses along with their pollen pellets, while the study included 65 worker honey bees (Singh *et al.* 2010). The same study also experimentally demonstrated that IAPV could be transferred between honey bee and bumble bee (*B. impatiens*) colonies contained within the same greenhouse and foraging on the same flowers, with both evidence of transfer from honey bees to the bumble bees and vice versa though the tracking of distinct IAPV variants (Singh *et al.* 2010). Singh *et al.* (2010) also showed that frames containing stored pollen with detected DWV can be infective to honey bees, but this does not demonstrate that pollen collected by bees prior to storage is also infective because viruses could be transferred from bees to pollen in the process of pollen storage. Another study showed that DWV extracted from pollen collected from flowers and forager honey bees can be infective to honey bees when injected directly into the bees (Mazzei *et al.* 2014). Although this study does not use field-realistic protocols, it does support the hypothesis that pollen is a mode of virus transfer and viruses from pollen can still be

infective to foraging bees. More research is needed to understand the role of pollen and shared floral resources in virus transmission between honey bees and wild bees.

Bee virus transmission risk factors

Along with agriculture and habitat loss, a variety of factors may contribute to virus transmission between managed and wild bees. Risk could differ spatially, for example, when comparing areas differing in agricultural intensity or floral metrics, but it could also differ temporally. Viral dynamics in honey bee hives are known to change over the course of the season in temperate regions, and they are often not consistent between viruses (Tentcheva *et al.* 2004; de Miranda *et al.* 2013; Antúnez *et al.* 2015; Robson-Hyska 2017; D’Alvise *et al.* 2019; Wham *et al.* 2024; Molinatto *et al.* 2025). Honey bee populations in hives also change over the course of the summer, starting low in the spring with a few thousand workers, and increasing up to 60,000 individuals by late summer (Farrar 1937; Lee and Winston 1987). Population increases could influence pathogen spillover, since more individuals out foraging likely results in more individuals from a hive interacting with other bees and the environment. In Manitoba, honey bee robbing in late summer when populations are high following canola bloom could also contribute to transmission risk, as bees steal nectar and honey from neighbouring hives (Peck and Seeley 2019). During this time, more interactions between colonies could increase pathogen spread, and newly infected bees, especially those weakened by other pathogens, may then deposit viruses on flowers in the landscape that other bees could acquire (Peck and Seeley 2019). Bees could also be foraging on a limited number of flowers in bloom during this time, increasing interactions and transmission risk.

Disease risk could also differ between bees depending on their social structure. In eusocial colonies, bees have more opportunities to interact with one another than in solitary

nests, which could facilitate the spread of pathogens (Dalmon *et al.* 2021; Figueroa *et al.* 2021; Tiritelli *et al.* 2024). Kleptoparasitic bees may face an even greater risk, as they enter and interact with individuals from multiple nests, and one kleptoparasitic species may interact with multiple host bee species (Bogusch *et al.* 2006; Manley *et al.* 2015; Lhomme and Hines 2019; Tiritelli *et al.* 2024). Little literature has examined these risks, but one study found a greater risk of DWV and ABPV transfer among kleptoparasitic bees than among other socialites, and kleptoparasitic wasps have been shown to share pathogens with their hosts (Tiritelli *et al.* 2024; Ranalli *et al.* 2026). Conversely, this study did not find an increase in risk for eusocial bees compared to solitary bees, which was similar to results from a study by Dalmon *et al.* (2021) but they oddly did observe an increased risk in some solitary nesting types (Tiritelli *et al.* 2024). Transmission risk could differ between the sexes, as female bees spend more time collecting pollen to feed their young, while the pollen that male bees interact with is primarily for their consumption (Thorp 2000; Ne’eman *et al.* 2006), although one study looking at the effect of sex on multiple non-viral pathogens in wild bees found no significant effect of sex (Figueroa *et al.* 2021).

Study system: Southern Manitoba

Southern Manitoba is comprised primarily of prairie, including tall-grass prairie, mixed-grass prairie, and Aspen Parkland (Reichman 1987; Smith *et al.* 1998). This is a region characterized by intensive agriculture due to the favourable environmental characteristics, flat terrain, and productive soils (Environment Canada 1996; Smith *et al.* 1998; Ojo and Manaigre 2021). Despite this, there are small areas of restored prairie, and larger patches of semi-natural habitat are present east of Winnipeg where the boreal plains ecozone begins (Environment Canada 1996; Smith *et al.* 1998; McLachlan and Knispel 2005). In Manitoba, the top crop by

area in 2024 was canola, with 3,308,200 acres harvested (Statistics Canada 2025). When in bloom, canola produces nectar and high quality pollen that honey bees and other bee species can forage upon (Pernal and Currie 2000; Cook *et al.* 2003). The high density of canola combined with the long days, warm temperatures, and little clouds or rain during the summer makes Manitoba, along with other prairie provinces, one of the most productive provinces in terms of honey production per hive, with 175 and 133 pounds of honey per colony produced in 2023 and 2024 respectively (Crane 1966; Emsen *et al.* 2014; Hoover and Ovinge 2018; Statistics Canada 2024). The primary type of canola production in Manitoba is commodity canola, used for oil and meal, which is planted from hybrid canola seed produced by crossing two parental lines, primarily produced in Southern Alberta (Clay 2009; Ovinge and Hoover 2018; Robinson *et al.* 2023). Commodity canola benefits less from insect pollination and is instead mainly self-pollinated compared to hybrid canola seed, which requires cross-pollination (Ovinge and Hoover 2018; Robinson *et al.* 2023). As a result, the main source of income for Manitoban beekeepers is from honey production as opposed to pollination, and honey bee hive stocking rates in crops may be lower in Manitoba than in areas where honey bees are relied upon for their pollination services, including Southern Alberta (Clay 2009; Robinson *et al.* 2023; Government of Manitoba 2025). Southern Manitoba is also a northern temperate region with somewhat harsh winters that can negatively impact honey bee overwintering survival, and these winters also impact disease dynamics when compared to regions lacking a winter season (Beaurepaire *et al.* 2020). Additionally, Manitoba includes intersecting prairie and boreal ecozones which provide diverse habitats, and it is centrally located within Canada (Smith *et al.* 1998; Gibbs *et al.* 2023). With this combination of both eastern and western species, a large diversity of bee species have been recorded for the province, totalling about 400 species records (Gibbs *et al.* 2023). This

combination of a common and rewarding crop for pollinators, a substantial beekeeping industry, both areas of intense agriculture and semi-natural habitat, and a large diversity of wild bee species makes Southern Manitoba an ideal study system to investigate pathogen spillover between honey bees and wild bees.

Study objectives

This study has two main objectives, the first is to identify the factors that impact virus spillover from honey bees to wild bees in agricultural and non-agricultural landscapes. This was tested by collecting honey bees and wild bees across 20 sites in Southern Manitoba varying in agricultural and honey bee intensity. From this objective are multiple research questions pertaining to the direction and significance of various factors:

1. Are honey bees transferring viruses to wild, unmanaged bees? If they are, one would predict that honey bee abundance and honey bee viral abundance (average viral gene copies across all honey bees tested) at a site will result in increased virus detections in wild bees. One would also expect that as honey bee populations in colonies grow over the course of the summer, virus transfer risk will increase due to increased interactions with wild bees. Sequences from honey bees and wild bees would also not be expected to cluster based on host species in a phylogenetic tree if there is continued spillover, but rather based on location as seen in other studies (Murray *et al.* 2019; Dalmon *et al.* 2021). Additionally, if virus spillover is negatively impacting wild bees, wild bee diversity would be expected to be lower at sites with a greater viral abundance in honey bees, when accounting for other factors.
2. Do sites with homogenous floral resources have increased virus transfer due to a greater overlap of floral resources used by honey bees and native bees? If so, wild bees at

agricultural sites will have a higher chance of virus detection. Factors including floral species richness and floral abundance may also have a negative effect on virus presence by reducing resource overlap between wild bees and honey bees.

3. Do wild bee species differ in their viral prevalence? One would expect eusocial and kleptoparasitic bees to have an increased risk of virus transfer due to increased interactions within colonies. Female bees may also be more likely to have detectable viruses due to increased pollen collection compared to males due to the presence of viruses on flowers. One would also expect bee species more closely related to honey bees to be at an increased risk, mirroring the patterns of virus sharing seen in vertebrates (Wells *et al.* 2020).

The second objective was to investigate the role of pollen and shared floral resources in virus transfer within bee communities, studied by testing pollen samples collected from bumble bees for viruses. From this objective came two main research questions:

1. Are wild bees collecting pollen with viruses deposited by the other bees present at each site? If this is true, sites with a higher abundance of viruses in honey and wild bees will yield collected pollen with a higher chance of virus presence.
2. Are viruses deposited on flowers by bees not through saliva or nectar regurgitation, but through another pathway? The hypothesized route of viruses to pollen was previously thought to be through saliva used to pack pollen together for transport, but fecal deposits or another pathway related to pollinator traffic on flowers appear to have stronger support, at least in honey bee foragers (Singh *et al.* 2010; Colwell *et al.* 2025). If this is the case, virus presence within pollen samples will be better predicted by the overall viral abundance

at the site where the bee and pollen were collected compared to the predictive power of the viral quantities in the pollen's respective bee.

METHODS

Field protocols

Site selection

For this project, ten agricultural canola and ten semi-natural sites (for a total of twenty sites) with a range of honey bee densities were selected to observe the effects of land use on virus transfer risk (Figure 1). Field sites were selected through collaboration with local beekeepers and farmers in southern Manitoba. Sites were separated by at least 9km to ensure there was no overlap of honey bee foragers from the same hive visiting multiple sites (Couvillon *et al.* 2015). Sites without honey bee colonies within 1.5km were considered low density, but ideally sites did not have colonies within 3km (Couvillon *et al.* 2015; Danner *et al.* 2016). This resulted in roughly four site types, with five of each type: high honey bee + agricultural, high honey bee + non-agricultural, low honey bee + agricultural, and low honey bee + non-agricultural. The purpose of including high and low-density sites was not to impose strict treatments, but to ensure a range of honey bee densities across sites. This variation was ideal as honey bee abundance was measured at each site and used in the models during analysis. Because of this, we had both a range of agricultural intensities and honey bee densities across our field sites (Table 4).

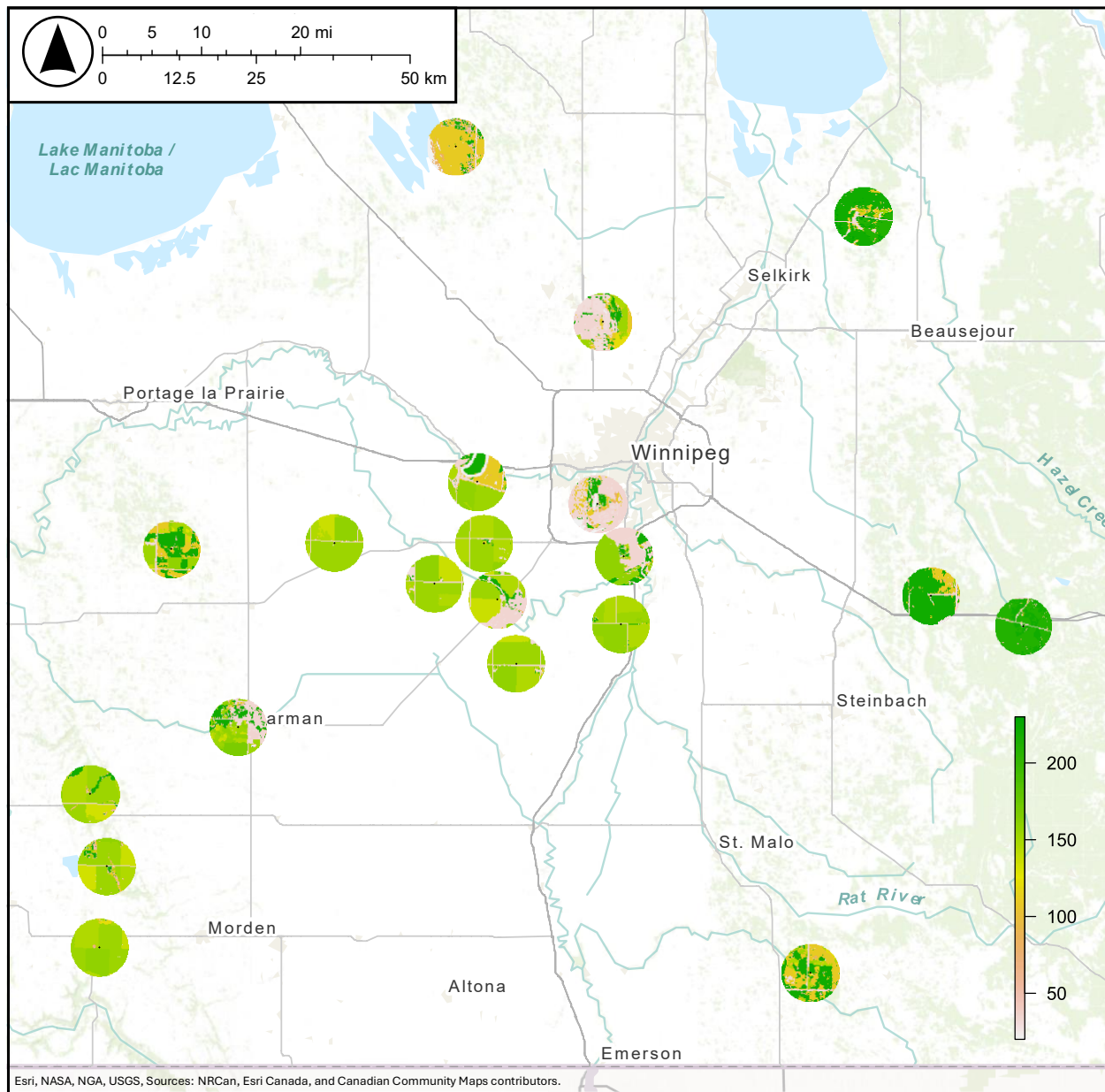


Figure 1: Field sites sampled in 2024. Each site is labeled with a plot of the landscape in the 1km radius surrounding each site (not to scale). Plot colours correspond to land use codes used by the Annual Crop Inventory listed in Table S1 (Agriculture and Agri-Food Canada 2024). Roughly, codes 20-35 include water, exposed land, and urban land use, codes 50-110 include shrub, grass and wetlands, codes 122-177 are for agricultural land use, and codes 210-230 are forests.

Sample collection overview

Samples were collected three times in 2024 between June and August. Sampling did not occur on rainy days, and sunny days with low wind speeds were favoured as sampling days. Sampling only occurred if temperatures were above 16°C, wind speeds were below 5m/s, some sun was present, and if any smoke was present, it did not appear to impact the activity of bees. A Kestrel Weather Meter was used to record weather conditions at each site (temperature, wind speed), and cloud cover and any smoke was noted. Sampling at sites occurred once before canola bloom (June 14 – 30), once during bloom (July 4 – 19), and once following bloom (July 29 – August 19), even in non-agricultural sites to ensure sampling time was not confounded with site. To check whether sites were at the correct stage for sampling, the Copernicus Browser (European Commission 2024) was used to view satellite images taken every few days, allowing the growth stage of each canola field. Wild bees and honey bees were collected via aerial net sampling using two methods: timed collection along transects and sampling unrestricted from transects.

Transect sampling (Abundance and diversity)

Transect sampling was performed approximately 250m from hives (Couvillon *et al.* 2015), if they were present at the site and their location was known, along two 50m transects, with each transect being sampled for 10 minutes. These transects were chosen at each site at each sampling event by surveying all areas in bloom (250m from hives when present), then choosing two transects that crossed through the areas with the highest densities of flowers (Figure 2). During canola bloom at agricultural sites, transects were placed in the canola field near where sampling round 1 transects were placed, with one transect approximately 1 meter into the field, and the other approximately 20 meters in, to get measurements with both an effect from the field

edge, which often increases bee abundance, and with a reduced impact from the field edge (Robinson *et al.* 2023; Killewald *et al.* 2025). When canola fields were not in bloom, transects were placed on field edges or in flower patches near/bordering the fields. An effort was made to sample and place transects in the same general area each sampling round. These transects were recorded for future reference using the phone application Map Marker (Figure 2C, 2D), which was also used to measure and visualize a 250m radius around any hives present (Biero 2024).

When sampling, a stopwatch was used to keep track of how much time had passed, and it was paused after a bee was netted and started again once it was properly stored in a 50mL cyanide kill tube. Any bees found were collected, including both wild bees and honey bees, and used to calculate honey bee density and wild bee diversity at each site. Because three people sampled over the course of the field season, each person sampled two of the six total transects at each site, with each of the three possible combinations of samplers occurring once per site to reduce sampler bias.

When processing samples, honey bees from each transect were counted then stored in 70% ethanol in labeled 30mL tubes. Wild bees were pinned and identified.

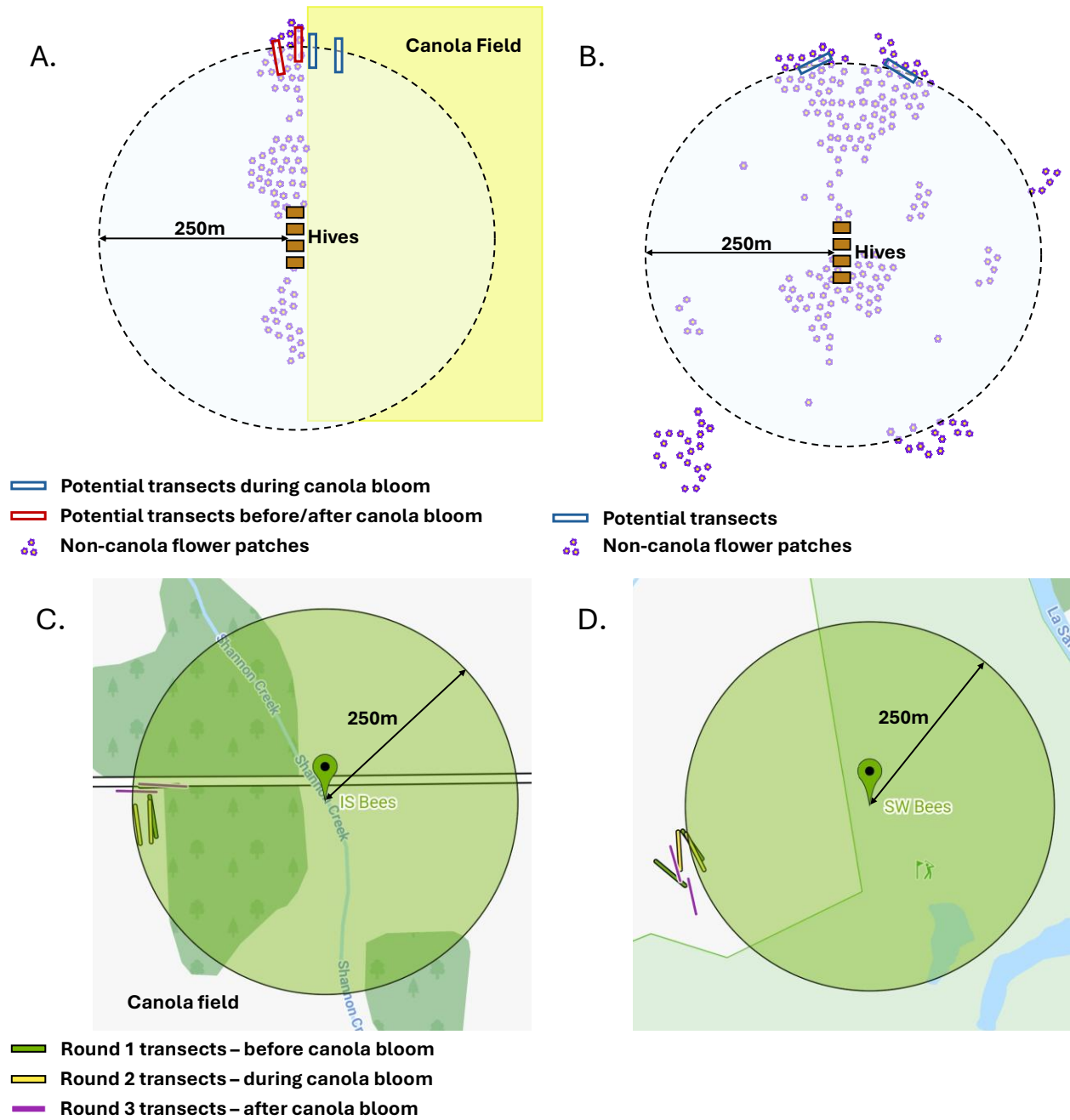


Figure 2: Transect sampling setup. Potential 50 meter transects at canola (A) and non-agricultural (B) sites with honey bee hives. Actual 50 meter transects placed at example canola (C) and non-agricultural (D) sites, as recorded during sampling, with bee hives labeled with green markers. Two 50 meter transects per site were selected for sampling at each of the three sampling events.

Unrestricted sampling (Viral analysis)

Aerial net sampling was also used to collect ten wild bees and ten honey bees, when present, from each site for viral analysis. Net bags secured with Velcro® that could be easily removed were used, and net bags were switched out between sites. Net bags were washed with hot water (to degrade viruses) and Dawn blue dish soap (for general cleaning) before being reused at a different site to reduce virus and pollen cross-contamination between samples. When possible, an effort was made to catch wild bees with visible pollen directly into tubes.

At each site, five specimens of the two most abundant wild bee species/groups (genus or subgenus, if identifying individuals to species was not possible) opportunistically sampled, with the caveat that the second group must be from either a different family (to compare susceptibility between Apidae, the family including honey bees, to other families) or of a different sociality (eusocial, solitary, or kleptoparasitic). If the two most common groups did not meet these criteria, the most common group was collected along with the next most common group that met these criteria. If it was not possible to find five individuals of each group, we first attempted to catch five closely related bees (from the same genus or family). If we could not do this, then 10 wild bees of any species were collected. This sampling pattern was performed to ensure that not only bumble bees would be caught, since they were often common, and so that we would limit the number of single species samples over the course of the field season. If we were unable to catch 10 wild bees on a particular day, we returned to the site as soon as possible to catch the remaining bees if time permitted (Fürst *et al.* 2014). To aid in identification, a reference specimen was caught and pinned if a species used for the viral analysis was not also caught in a transect. The flower species that each bee was collected on was also recorded (identified or

described to the best of our abilities), then later morphologically identified based on iNaturalist observations made at the site (see Data analysis – Floral diversity).

Honey bee and wild bee samples were used for viral analysis, so to reduce cross-contamination, they were collected into individual 30mL sterile tubes labeled with a sample number, site, date, and whether the sample was a wild bee or honey bee. On a datasheet, date, time, site, weather, sample number, flower species, pollen presence (Y/N), and initial identification information were recorded for each collected bee. Samples were kept on ice in a cooler during transportation, then frozen and stored at -80°C upon return to the lab until identification and pollen collection were performed.

Identification of transect samples

Wild bees collected for diversity measures were kept in a -20°C freezer until they could be pinned/pointed and identified. Samples were pinned and labeled, then morphologically identified under either a Nikon SMZ745, SMZ800N, or SMZ18, and using a combination of the resources listed in Table 1, unpublished keys created by Micheal Arduser and Dr. Joel Gardner, reference specimens from the J. B. Wallis/R. E. Roughley Museum of Entomology, and assistance from bee taxonomist Dr. Jason Gibbs. These samples were also used as references to help efficiently identify wild bees used for viral analysis. Specimens were deposited into the J. B. Wallis/R. E. Roughley Museum of Entomology.

Viral analysis

For viral analysis, all bee samples were processed individually for consistency and to collect high resolution prevalence data. Molecular methods described here follow those

performed by Sharma (2025) and the general procedure is typical of the field (de Miranda *et al.* 2013). The methods are described in full detail here.

Pollen removal, identification, and cleaning of samples

Wild bees collected for viral analysis were identified on cold plates. Before and after identification, bees were transported and kept in liquid nitrogen to keep them frozen when not stored at -80°C. Cold plates consisted of an insulated container filled with ice, with a metal plate placed on top, and a Kimwipe™ placed on the metal. This was placed under a Nikon SMZ745 which was used for identification. Between every bee, the metal plate and any forceps used to handle the bees were cleaned with 70% ethanol followed by RNaseZap™. A new Kimwipe™ was placed on the metal plate, and a bee in its tube was removed from the liquid nitrogen and placed on the Kimwipe™.

Prior to the identification of each bee used for viral analysis, pollen was removed from each bee while it was on a cold plate immediately after it was removed from liquid nitrogen. Any pollen present on the bee was removed via forceps and placed in a labeled 1.5mL microcentrifuge tube, which was immediately placed back in liquid nitrogen. The approximate relative size of the pollen load (XS-XL) was recorded to assist with choosing pollen samples to later process.

Next, the bee was identified using the keys listed in Table 1, unpublished keys by Micheal Arduser and Dr. Joel Gardner, and by comparing it both to pinned specimens caught during the summer in transects, and specimens from the J. B. Wallis/R. E. Roughley Museum of Entomology as a reference collection. Images were taken through the microscope of the bee in positions showing important identification features. This was to assist with verifying identifications later, and to have a type of voucher for each sample. Images of difficult to identify

specimens were examined and corrected by bee taxonomist Dr. Jason Gibbs, and specimens with uncertain identifications were denoted with “c.f.”. After identification and imaging, the bee was placed back into the original 30mL tube it was collected into, and 95% ethanol was added to the tube from a bottle kept on ice. The bee was then gently shaken and swirled with the ethanol in the tube to remove any excess pollen from the bee. The bee was then removed and placed back on the cold plate, and ethanol was dabbed off on the Kimwipe™. This washing step was repeated if pollen was still clearly visible. Once the bee was clean and mostly dry of ethanol, it was placed into a new labeled 1.5mL microcentrifuge tube (or 5mL screw-cap centrifuge tube for large bees) and placed back in liquid nitrogen. Samples were stored at -80°C until processed for viral analysis.

Table 1: Published resources used to identify bees. For unlisted taxa, the resources used are in the last row and/or are unpublished. Unpublished keys are not included.

Group(s)	Keys Used
Augochlorini	(Portman <i>et al.</i> 2022)
<i>Andrena</i>	(Ribble 1968; LaBerge 1971, 1973, 1986; Bouseman and LaBerge 1978)
<i>Bombus</i>	(Lavery and Harder 1988; Williams <i>et al.</i> 2014; Milam <i>et al.</i> 2020)
<i>Ceratina</i>	(Rehan and Sheffield 2011)
<i>Coelioxys</i>	(De Silva 2012)
<i>Epeolus</i>	(Onuferko 2017)
<i>Hylaeus</i>	(Oram 2018)
<i>Lasioglossum</i>	(McGinley 1986; Gibbs 2010; Hettiarachchi and Gibbs 2024)
<i>Megachile</i>	(Sheffield <i>et al.</i> 2011)
All/Other groups	(Mitchell 1960, 1962; Packer <i>et al.</i> 2007; Gibbs <i>et al.</i> 2023; Ascher and Pickering 2025)

Honey bees were processed in the same way, except removed pollen was not saved, identification was not necessary, and no images were taken as honey bees can be accurately identified in the field.

Homogenization

Homogenization was performed using a mortar and pestle. Samples were removed from the -80°C freezer and transferred to a dewar with liquid nitrogen. Samples were added to a sterilized mortar with some liquid nitrogen and crushed with a sterilized pestle. For small bees, a smaller mortar and pestle were used. Up to 0.1mL of homogenate was then transferred to a labelled 1.5mL microcentrifuge tube and placed back in the dewar with liquid nitrogen. If the homogenate was greater than 0.1mL, then any extra was placed in a labeled 2mL microcentrifuge tube. Samples were then weighted before being either immediately processed or stored at -80°C until RNA extraction was performed. Any extra homogenate was kept as backup at -80°C.

RNA extraction

RNA extraction was completed using a Qiagen RNeasy Kit following manufacturers instructions. 700uL of Buffer RLT (Qiagen), which included 450μL of 2-mercaptoethanol per 45mL of buffer, was added to each microcentrifuge tube with a sample, unless the weight of homogenate was less than 0.05g, then 650uL was added instead. Each sample was then dipped in liquid nitrogen until it turned white (about 5 seconds). The samples were thawed at room temperature and mixed by repeatedly, slowly inverting them by hand for 8 minutes in a tube rack, followed by centrifugation (8 minutes at 13,000 rpm). 600uL of the supernatant was then pipetted into a new 1.5mL Fisherbrand microcentrifuge tube. 600uL of 100% isopropanol was added to the supernatant, and each tube was immediately gently inverted until mixed. From this, 600uL was transferred to an RNeasy spin column (Qiagen) which was then centrifuged (15 seconds at 12,000 rpm). The flow through was then discarded. The remaining 600uL of sample was again transferred to the spin column, centrifuged (15 seconds at 12,000 rpm), and the flow

through was discarded. 700uL of RW1 buffer (Qiagen) was then added to the sample in the spin column, which was then centrifuged (15 seconds at 12,000 rpm) and the flow through was discarded. 500uL of RPE buffer (Qiagen) was then added to the spin column which was then centrifuged (15 seconds at 12,000 rpm), and the flow through was discarded. The RPE buffer step was then repeated. The collection tube of the spin column was then discarded, and the spin column was placed in a new 2mL collection tube. This was centrifuged (2 minutes at 13,000 rpm) to dry the membrane, and then the collection tube was discarded. The spin column was placed in a new 1.5mL microcentrifuge tube (labeled as RNA) and 50uL of molecular grade water (MGW) was added to the centre of the column and incubated for 4 minutes at room temperature. To elute RNA, the sample was then centrifuged (3 minutes at 13,000 rpm) then checked to ensure that the 50uL was at the bottom of the collection tube, and then the spin column was discarded.

A spectrophotometer (Thermo Scientific NanoDrop™ 8000) was used to measure the concentration of RNA in each sample to calculate the volume of RNA to use for complementary DNA (cDNA) synthesis. It was also used to check for sample contamination, and all samples were confirmed to have a 260/280 of greater than 2.00 (Rapley and Heptinstall 1998). The spectrophotometer was blanked with molecular grade water (MGW), and samples were analysed in duplicate using 1.5µL of extract. The RNA extracts were then kept on ice and immediately used to synthesize cDNA.

cDNA

For cDNA synthesis, the concentration of each RNA extract was calculated from the spectrophotometer measurement and 1ug of RNA was added to MGW for a combined volume of 16µL. 4µL of iScript™ Reverse Transcription Supermix (BioRad) was added for a 20µL total

volume in PCR tubes. Complementary DNA production was performed in duplicate reactions, so 40µL of cDNA was produced per sample. Each sample was mixed well by pipetting up and down until the solution was homogenous following the addition of iScript™, then centrifuged for 1 minute at 4680 rpm to remove air bubbles from the samples. The samples were then placed in the thermocycler (DNA Engine® Peltier Thermal Cycler BioRad) using the cDNA protocol of 5 minutes at 25°C, 20 minutes at 46°C, and 1 minute at 95°C. The samples were then removed and centrifuged to reduce condensation. Samples were then transferred to 1.5mL microcentrifuge tubes, combining the cDNA duplicates with 20µL of MGW to produce 60µL of 2:1, cDNA:MGW diluted cDNA for qPCR.

RT-qPCR

Hard-Shell BioRad 96-well plates were used for real time-quantitative polymerase chain reaction (RT-qPCR) analysis. Each plate included wells with SsoFast EvaGreen® Supermix (10µL per well) (BioRad), MGW (7µL per well), primers (forward & reverse, 1µL per well total), and 2µL of sample cDNA or gBlocks™. Primers (Integrated DNA Technologies) were prepared ahead of time, diluted in 1× TE buffer (pH 7.5) to 5µM of each the forward and reverse primer in the primer mix, and stored at -20°C. Synthetic gBlocks™ (Integrated DNA Technologies) were used to construct the standard curves used to quantify each virus (10⁸-10⁴ gene copies/µL), and they were diluted 1:1 with 1× TE buffer (pH 7.5), so 2µL were used per well. Each sample was tested for five viruses commonly found in Manitoba: DWV-A, DWV-B, IAPV, BQCV and SBV, and each sample was tested in triplicate (Robson-Hyska 2017; Sharma 2025). Virus primers with their sources are listed in Table 2, and gBlocks™ are the same as those used and listed by Sharma (2025). After testing multiple potential reference genes across bee species, S28 (a ribosomal gene) was chosen as an internal control (see Methods and Results

for Internal controls in RT-qPCR). One S28 well per sample was included as an internal control, and negative controls (one well each) were performed for EvaGreen® with MGW, and EvaGreen® with MGW and each primer mix used on the plate (in place of virus samples and primers, additional MGW was added to each well). Wells were filled via pipetting in a UV hood, and the plate was sealed with a microseal (BioRad Microseal 'B' PCR Plate Sealing Film) following the addition of all reagents. The plate was then centrifuged (1 minute at 4680 rpm) and placed in the thermocycler (CFX96, BioRad). The following PCR protocol was used according to the manufacturer's instructions: 95°C for 30 seconds, then 40 cycles of 95°C for 5 seconds then 55°C for 5 seconds, followed by a melt curve step from 65°C to 95°C, with an increase of 0.5°C per cycle and a ramp rate of 0.5°C/second.

Results were output as a digital file and analyzed via Bio-Rad CFX Manager™ (v 3.0), with baseline thresholds auto calculated by the program. Results were in the format of gene copies per 0.067µg of RNA but were converted to gene copies per 1µg of RNA for tables and graphs made for the data summary (Figure 4B, 12B) for clarity. The raw output in gene copies per 0.067µg of RNA was still used for modeling and comparing intensities, and the log of this value was taken for all analyses as the log of gene copy values is required when analyzing honey bee viruses (de Miranda *et al.* 2013). Each gene copy only indicates presence, and is not confirmed to be infective (de Miranda *et al.* 2013). Only one virus was tested per plate, with up to 22 samples per plate. Results were checked to ensure that sample melt curves matched those of the standards. Melt curves that were clearly off target detections only seen in a specific wild bee species/group were excluded from analysis, and other restrictions were made based on sequencing results (see Virus sequencing results). Plates were stored at -20°C so that PCR product could be later sequenced from select samples to confirm sequence identity.

Table 2: List of primers used in qPCR for viral analysis

Primer name abbreviation	Sequence (5'-3')	Product size (bp)	Target area*	References
DWV-A F	TTCATTAAAGCCACCTGGAACATC	136	Just before RdRp, in ORF	Locke <i>et al.</i> 2012
DWV-A R	TTTCCTCATTAACCTGTGTCGTTGA			
DWV-B F	TATCTTCATTAACCAACGCCAGGCT	140	Just before RdRp, in ORF	McMahon <i>et al.</i> 2015
DWV-B R	CTTCCTCATTAACCTGAGTTGTTGTC			
IAPV F	CCATGCCTGGCGATTAC	203	Beginning of ORF2, just before a capsid	Locke <i>et al.</i> 2012, Robson-Hyska 2017
IAPV R	CTGAATAATACTGTGCGTATC			
BQCV F	CCTGTATTCATGCATCTCAGA	310	Following a capsid in ORF2, partially in 3'UTR	Robson-Hyska 2017, Chen pers. comm.
BQCV R	GCAACAAGAAGAAACGTAAACCAC			
SBV F	TTGGAACCTACGCATTCTCTG	335	Following a capsid, in ORF	Locke <i>et al.</i> 2012, Robson-Hyska 2017
SBV R	GCTCTAACCTCGCATCAAC			

*Based on GenBank reference sequences – BQCV: NC_003784.1, SBV: NC_004830.2, DWV-B: NC_006494.1, DWV-A: NC_004830.2, IAPV: NC_009025.1

Internal controls for bees in RT-qPCR

Although actin can be used as a reference gene for honey bees, it has been unsuccessful in more distantly related species (Levitt *et al.* 2013; McMahon *et al.* 2015). To find a reference gene that could be detected in honey bees and most wild bees, multiple primers were tested across a range of species, using extra field samples that were not needed for analysis. The bee species tested were: *Apis mellifera*, *Bombus rufocinctus* Cresson, 1863, *Bombus ternarius* Say, 1837, two *Andrena* spp., *Lasioglossum zonulus* (Smith, 1848), *Lasioglossum lecozonium* (Schrank, 1781), *Halictus rubicundus* (Christ, 1791), and *Hoplitis albifrons* (Kirby, 1837). These bees included four of the five bee families commonly found in Manitoba, with only the family Colletidae missing. The primer combinations tested are listed in Table 3.

Table 3: List of primers tested as reference genes for bees

Primer abbreviation	Sequence (5'-3')	Organism of origin	References
β -Actin F	AGGAATGGAAGCTTGCG GTA	<i>Apis mellifera</i>	Chen <i>et al.</i> 2005
β -Actin R	AATTTTCATGGTGGATG GTGC		
S28 F	AGCGCGGATATCTTGGA CTG	<i>Bombus terrestris</i>	Sankar <i>et al.</i> 2024
S28 R	TCAAGACGGGTCCTGAG AGT		
S18 F	AGCGTGCTGGAGAATGT TCA	<i>Bombus terrestris</i>	Sankar <i>et al.</i> 2024
S18 R	TCGTTCCAAGTCCTCACG AAG		
Opsin F	TTCGAYAGATAACAACGT RATCGTNAARGG	Colletidae	Mardulyn and Cameron 1999, Almeida and Danforth 2009, Radzevičiūtė <i>et al.</i> 2017
Opsin R	ATATGGAGTCCANGCCA TRAACCA	<i>Drosophila pseudoobscura</i> Frolova, 1929	
CO1 F	GGTCAACAAATCATAAA GATATTGG	Universal	Folmer <i>et al.</i> 1994, Radzevičiūtė <i>et al.</i> 2017
CO1 R	TAAACTTCAGGGTGACC AAAAAATCA		
GAPDH F	GCTGGAGCTGAATATGT TGTAAGATC	<i>Bombus terrestris</i>	Sankar <i>et al.</i> 2024
GAPDH R	AGTAGTGCAGGAAGCAT TAGAGATAACT		
GAPDH HB F	ACCTTCTGCAAAATTAT GGCGA	<i>Apis mellifera</i>	Reim <i>et al.</i> 2013
GAPDH HB R	CACCTTTGCCAAGTCTA ACTGTTAAG		

Viral analysis of pollen

Only pollen samples collected from bumble bees labeled as medium or larger were processed. Viral analysis of pollen samples was nearly identical to the protocol used for bees. No protocol changes were noted by Singh *et al.* (2010) when they performed this analysis on pollen

samples. For pollen samples, the smaller-sized mortar and pestle were always used due to the small size of samples and following homogenization the homogenate was returned to the 1.5mL microcentrifuge tube in which the pollen was originally stored. The other main difference in protocols was that for RT-qPCR, no internal control was used for each pollen sample, but gBlocks™ were still used as a positive control for the plate and for the standard curve, and gene copy numbers were still normalized to RNA concentrations. Because no internal control was used, more samples could be fit on each plate.

Data analysis

All analyses were conducted using R 4.4.3 (R Core Team 2025) and RStudio 2025.5.1.513 (Posit team 2025), except for the landscape metrics analyses as discussed below. All generalized linear mixed models (GLMMs) were created using the function “glmmTMB” from the package “glmmTMB” (McGillucuddy *et al.* 2025). Plots of fixed effects were performed using the function “ggeffect” from the package “ggeffects” with the margin argument set to “empirical” (Lüdtke 2018). For each analysis, an alpha level of 0.05 was used.

Prevalence and intensity comparisons, and definitions

For all analyses, virus measures in a group of bees are referred to as prevalence, intensity, or abundance. Prevalence is calculated as the proportion of bees detected with a particular virus. Intensity, sometimes referred to as mean intensity, is calculated as the average number of viral gene copies (calculated as gene copies/μg RNA, and the average is taken from the log value) in bees detected with the virus, excluding negative samples. Abundance can be calculated in two ways which give identical values: the average number of viral gene copies across all bees, including those with no virus detected, or prevalence × intensity. All terms are borrowed from

their common use in parasitology (Margolis *et al.* 1982; Murray *et al.* 1996; Oorebeek and Kleindorfer 2008).

Comparisons of virus prevalence between honey bees and wild bees were performed using methods described in Fleites-Ayil *et al.* (2023), where a GLMM with a binomial distribution was constructed with virus presence/absence as the response variable. Bee identity (honey bee or wild bee) was the only fixed effect, and site was included as a random effect. Model assumptions were tested using the function “simulateResiduals” from the package “DHARMA”, and no violations were detected (Hartig 2024). Because BQCV prevalence in honey bees was 100%, Fisher’s exact test was instead used to compare prevalence using the function “fisher.test”.

Comparisons in virus intensity between honey bees and wild bees were performed as an evaluation of susceptibility and host suitability between these two groups. This was done using either a Welch's t-test using the function “t.test”, or a Mann-Whitney U test using the function “wilcox.test”, depending on whether the distributions of these data were normal or not. The viral intensity, which is the average gene copies (calculated as the log of gene copies per 0.067µg RNA + 1) across all infected bees, was calculated separately for wild bees and honey bees caught at the same site on the same date. For each group, honey bees and wild bees, the mean intensity was calculated at the site level, treating each date as a subsample. These values of the mean virus intensity were then used to compare honey bee and wild bee intensity for each virus.

Phylogenetic distance

Because bees from across five different families were collected and tested, we had the opportunity to test the effect of phylogenetic distance from honey bees on virus susceptibility.

Phylogenetic data for each species were obtained from BeeTree, a publicly available bee phylogeny (Henríquez-Piskulich *et al.* 2024). In a few cases, a closely related bee species was substituted when a species not available in the database, but this made no difference in distance measurements as closely related bee species had equal phylogenetic distances from honey bees. The species substituted were *Stelis montana* Cresson, 1864 for *Stelis coarctatus* Crawford, 1916, *Protandrena aestivalis* (Provancher, 1882) for *Protandrena perlaevis* (Cockerell, 1896), *Hylaeus saniculae* (Robertson, 1896) for *Hylaeus fedorica* (Cockerell, 1909), *Hylaeus modestus* Say, 1837 for *Hylaeus gaigei* (Cockerell, 1916), *Nomada lehighensis* Cockerell, 1903 for *Nomada composita* Mitchell, 1962, and *Lasioglossum admirandum* (Sandhouse, 1924) for the more general *Lasioglossum viridatum* group. A pruned set of 1000 bootstrap trees containing the species of interest was downloaded, then imported into R using the function “read.tree” from the package “ape” (Paradis and Schliep 2019). A maximum clade credibility tree was then made using the function “maxCladeCred” from the package “phangorn” (Schliep *et al.* 2017). To calculate pairwise distances, “ape::cophenetic.phylo” was used, and only distances between honey bees and each other bee species were retained for analysis (Paradis and Schliep 2019).

Sociality

Sociality data for each species were obtained from Gibbs *et al.* (2023). The categories of “solitary”, “eusocial”, and “parasitic” were used to simplify the analysis. Bees of the genus *Ceratina* are classified as subsocial, forming both social and solitary nests, but for simplicity, because they interact with other bees within their nests less than eusocial bees would, and because only bees of that genus would make up the category subsocial, they were included in the category “solitary” (Rehan *et al.* 2010; Mikát *et al.* 2022).

Landscape metrics

To calculate the agricultural land use surrounding each site in 2024, data were obtained from the Annual Crop Inventory provided by Agriculture and Agri-Food Canada in the form of a GeoTIF file (Agriculture and Agri-Food Canada 2024). Code used was similar to Killewald *et al.* (2024). Data were extracted from the file using the packages “raster”, “landscapemetrics”, “sampSurf”, and “rgdal” using R 4.3.1 and RTools 4.3, since “rgdal” has been retired as of the end of 2023 (Hesselbarth *et al.* 2019; Gove 2021; Bivand *et al.* 2023; Hijmans 2025). The land use in the 1km radius around each site was calculated, and the output was interpreted using the crop classifications .csv provided by the Annual Crop Inventory (Table S1). To calculate the percent of agricultural land use in a 1km radius around each site, the number of pixels in the area classified as agricultural was divided by the total number of pixels with a defined value. A full list of land use values per site can be found in Table S2. Semi-natural and urban classifications were initially considered as variables in analysis, but each was highly correlated with agriculture, so only the percentage of agriculture was used as a variable in models. Percent agriculture was included in models to test the effects of agricultural intensification and homogenous landscapes on virus transfer risk, including homogenous floral resources.

Floral abundance

Because virus transfer may be impacted by overlapping floral resource use by honey bees and wild bees, floral abundance was measured and included in the relevant models. To measure floral abundance at each site, images of two 1 meter $\times$ 1 meter quadrats, one at 15.66 to 16.66 meters and one at 33.33 to 34.33 meters, were taken along each of the two transects. A coin was flipped to determine which side of the transect the first quadrat would be placed on, and the second quadrat was placed on the side opposite to the first.

All images were analyzed in ImageJ by adjusting the colour threshold (hue, saturation, and brightness) to match each flower colour (each flower colour was individually analyzed then later summed) (Schneider *et al.* 2012). Then, the tool “analyze particles” was used on the selection inside the quadrat, and the pixel size and circularity were adjusted to best match the flowers present while filtering out noise. The options “show outlines” and “summarize” were selected, so that the analyzed particles could be visualized and saved, and so the percent area covered by flowers could be calculated. With flower colours difficult to separate from the background (ex: white flowers like white clover), sometimes the flowers needed to be manually selected and separated from the background with the polygon selection tool, then the total area taken up by the flowers, in pixels, was calculated as above using “analyze particles”, followed by manually calculating the percent area after selecting and measuring the size of the entire quadrat.

Floral diversity

All flowering species present at each site were photographed and uploaded to iNaturalist both for record-keeping purposes, to identify on which flower species each bee was foraging, and to calculate flowering species richness for each site as a measure of diversity. All plant records between June and August collected by all samplers were added to a project on iNaturalist.

To calculate the number of flowering species at each site, the package “rinat” was used to import records into R from the iNaturalist project (Barve and Hart 2022). A .csv file was made manually with the start and end times for each sampling site and round (sampling event), and the function “interval_join” from the package “fuzzyjoin” was used to assign a site and round to each observation (Robinson 2020). Observations that did not fall into the sampling times were removed, as some personal observations were automatically included in the project. All observations were checked to ensure they were identified to at least the genus. Potentially

problematic observations were identified by searching for any observations at the site and round that were identified only to the level of genus and were the same genus, to ensure that these observations were not different species from the same genus. Other problematic observations were identified by labelling any observations identified to the same genus, but to different levels of identification (ex: one identified to genus and one to species) within the same site and round. These were checked to make sure that different species had unique taxon names (so leaving them at different ranks could be acceptable if they were different species), and that the same species also shared the same name (often done by making sure they were identified to the same rank). The number of unique species names per site and round was then calculated to measure flowering species richness. This was also repeated at the site level for modeling wild bee diversity.

Modeling virus presence in wild bees

Modeling methods were similar to those used by Fürst *et al.* (2014) and Maurer *et al.* (2024), and were conducted using GLMMs using the function “glmmTMB” (McGillcuddy *et al.* 2025). For each global model, wild bee virus presence/absence was the response variable, a binomial distribution was used, and each virus was analyzed in its own separate model. Each wild bee was its own individual row within the data frame.

The global models for modeling wild bee (WB) virus presence included the fixed effects of honey bee (HB) virus abundance, honey bee density, percent agriculture, Julian date, sex, sociality, flowering species richness, and floral abundance as fixed effects. Wild bee species and site were each included as random effects:

$$\begin{aligned}
WB \text{ virus detected } (Y/N) \approx & \beta_1(HB \text{ virus abundance}) + \beta_2(HB \text{ density}) + \\
& \beta_3(\% \text{ Agricultural}) + \beta_4(\text{Phylogenetic distance from HB}) + \beta_5(\text{Day}) + \beta_6(\text{Sex}) + \\
& \beta_7(\text{Sociality}) + \beta_8(\text{Flowering species richness}) + \beta_9(\text{Floral abundance}) + \\
& (1|\text{Species}) + (1|\text{Site}) + \beta_0
\end{aligned}$$

Honey bee virus abundance was calculated as the average number of gene copies (in log of gene copies per 0.067µg RNA + 1) from all honey bees caught at the site during the sampling round in which the wild bee was caught. If no honey bees were present, this value was zero. Honey bee density was calculated as the number of honey bees caught per 10 minute transect, averaged between the two transects performed per site each sampling round. Julian date was calculated as the number of days since January 1st, 2024. Sex was the sex of the wild bee tested: either male or female. Percent agriculture, phylogenetic distance from honey bees, sociality, floral abundance, and flowering species richness were calculated as described above in their respective sections. All numeric variables were scaled using the function “scale”.

The “dredge” function from the “MuMIn” package was applied to the global model for each virus (Bartoń 2025). All models with a difference in Conditional Akaike Information Criterion value (ΔAIC_c) from the top model that was less than 2 were used to create a model average using the function “model.sel” from the package “MuMIn” (Bartoń 2025). These model averages were used to evaluate the significance and direction of effect of each variable on wild bee virus detection. Because of the low number of detections of DWV-A and IAPV in wild bees, analyses via modeling were not possible for these viruses. Conditional and marginal R-squared values were calculated using the function “r2” from the package “performance” (Lüdecke *et al.* 2021).

The collinearity of fixed effects was assessed using a VIF (Variance Inflation Factor) test using the function “check_collinearity” from the package “performance” for all global models and top models included in each model average (Lüdecke *et al.* 2021). All fixed effects in models had VIFs of less than 4. Additionally, global and top model assumptions were tested using the “simulateResiduals” function from the “DHARMA” package, and no violations were detected (Hartig 2024).

Initially, models were made using the number of gene copies (in log of gene copies per $0.067\mu\text{g RNA} + 1$) in each wild bee as the response variable, using a few different distributions including a zero-inflated negative binomial distribution, a negative binomial distribution, and gaussian distribution. None of these models met model assumptions when tested with the “DHARMA” package, so resorting to logistic regression with virus presence/absence in wild bees as the response variable was necessary. An interaction between honey bee virus abundance and honey bee density was also initially included in each global model, as this could be a plausible interaction, but because the interaction was insignificant for all viruses and its removal did not change the significance or sign of any main effects, it was removed to simplify the interpretation of the main effects.

Modeling wild bee diversity

Wild bee diversity was calculated per site from all wild bees caught in transects over the course of the field season, totalling six transect measurements per site (two each time the site was sampled). Diversity was calculated in R using the function “diversity” and the index “shannon” from the package “vegan” (Oksanen *et al.* 2025). To assess whether virus abundance in honey bees or other measured factors affect wild bee diversity, a global model of wild bee diversity was developed. The global model was made using the function “lm” and included virus

abundance of each virus in honey bees at each site, honey bee density, agricultural intensity, floral abundance, and floral species richness as fixed effects explaining wild bee diversity. The function “dredge” was applied to the global model, and a model average was created from all models with a $\Delta AICc$ of less than 2 to determine what effects were significant. The global model and top model were tested using the “simulateResiduals” function from the “DHARMA” package, and no violations were detected (Hartig 2024). A VIF test was conducted on the global model and top model using the function “check_collinearity” from the package “performance” (Lüdecke *et al.* 2021). R squared values were calculated using the function “r2” from the package “performance” (Lüdecke *et al.* 2021).

Modeling virus presence in pollen

To test whether viruses are deposited on flowers by the bees present at each site, an initial global model was created for each virus detected in pollen using a logistic regression (binomial) model. These included virus presence in pollen as the response variable, and virus abundance, agricultural intensity, and date as fixed effects, and site as a random effect.

$$\text{Pollen virus detected (Y/N)} \approx \beta_1(\text{HB and WB virus abundance}) + \% \text{ Agriculture} + \text{Date} + (1|\text{Site}) + \beta_0$$

Only these parameters were included due to limited samples, and because these parameters were most often significant when modeling virus presence in wild bees. Species was not included as a random effect because only pollen samples from *Bombus* were tested. Virus abundance was calculated as the average number of viral gene copies (in log of gene copies per 0.067µg RNA +1) detected in all bees caught at that site in that sampling round. This included all honey bees and wild bees, to account for all potential contributions to virus deposition on

flowers. Agricultural intensity was expressed as the percentage of agricultural land use in the 1km radius around each site. Date was calculated as the number of days since January 1st, 2024. Site was included as a random effect to account for multiple samples collected from the same site and was retained even when it caused a singular fit warning to account for this lack of independence between samples. All numeric variables were scaled using the function “scale”.

The “dredge” function from the MuMIn package was applied to the global model to determine which variables are significant in predicting virus presence in pollen (Bartoń 2025). All models with a delta AICc of less than 2 were then used to create a model average using the “model.avg” function from MuMIn, and only significant predictors in the full model average were retained for further analyses (Bartoń 2025). Conditional and marginal R squared values were calculated using the function “r2” from the package “performance” (Lüdtke *et al.* 2021).

Next, to test which is a better predictor of virus presence in pollen, either the virus abundance from all bees present at a site or the viral gene copies in the specific bee from which pollen was collected, candidate models were created and ranked using AICc. For each virus, a model was made containing pollen virus presence as the response variable, virus abundance from all bees at the site as a fixed effect, and site as a random effect. Another model was created with pollen virus presence as the response variable, number of viral gene copies (in log of gene copies per 0.067µg RNA + 1) in the bumble bee that pollen was collected from as a fixed effect, and site as a random effect. Because no other predictors were significant in the previous model average, none were included in these models.

$$\text{Pollen virus detected (Y/N)} \approx \beta_1(\text{HB and WB, virus abundance}) + (1|\text{Site}) + \beta_0$$

$$\text{Pollen virus detected (Y/N)} \approx \beta_1(\text{Individual, \# of virus gene copies}) + (1|\text{Site}) + \beta_0$$

A random effects model containing only site as a random effect and a null model were also created. All four models were ranked using the “model.sel” function from “MuMIn” (Bartoń 2025) to determine which model best predicts virus presence in pollen. Again, conditional and marginal R-squared values were calculated using the function “r2” from the package “performance” (Lüdecke *et al.* 2021).

To test whether models met model assumptions, the “simulateResiduals” function from the “DHARMA” package was used to assess all global models, top models, and candidate models, and no violations were detected (Hartig 2024). The collinearity of fixed effects was assessed using the function “check_collinearity” from the package “performance” for all global models and no issues were detected (Lüdecke *et al.* 2021). Initially, other model types were tested, but only logistic regression models did not violate model assumptions and passed the tests performed by “simulateResiduals”, so virus presence/absence was used as the response variable instead of the number of viral gene copies in pollen samples.

Virus sequencing and phylogenetics

Sequencing protocol

PCR products of select samples were sent for sequencing to confirm their sequence identity. Because there was a range of melt curve temperatures detected for most viruses, both in honey bees and wild bees, samples from each melt curve temperature were sequenced from both honey bees and wild bees when possible.

Plates containing the selected samples were thawed, and the microseal over all three triplicate wells of each selected sample was pierced. The 20µL of PCR product from each

triplicate well were pooled together and pipetted into a new 1.5mL microcentrifuge tube, for a total of 60µL of product.

A Monarch[®] Spin PCR & DNA Cleanup Kit was used to clean up samples for sequencing according to the manufacturer's instructions. 300µL of Buffer BZ was added to the 60µL of PCR product and mixed by pipetting up and down. Then, the mixture was added to the spin column and centrifuged for 1 minute at 13,000rpm, and the flow through was discarded. 200µL of Buffer WZ was then added to the spin column, which was centrifuged for 1 minute at 13,000rpm and the flow through was discarded, then this step was repeated. The spin column was then centrifuged again for 1 minute at 13,000rpm to ensure the membrane was dry, then transferred to a new 1.5mL microcentrifuge tube. 15µL of Buffer EY was added to the centre of the column, which was incubated for 1 minute at room temperature before being centrifuged for 1 minute at 13,000rpm to elute the sample.

A spectrophotometer (Thermo Scientific NanoDrop[™] 8000) was used to measure the concentration of DNA in the same way described for RNA (see RNA extraction above), except the DNA setting was selected instead of the RNA setting. To check for purity, each sample was confirmed to have a 260/280 between 1.80 and 2.00 (Miller *et al.* 1988). A dilution of each sample was made with a concentration of 50ng/µL, as required for sequencing. Sanger sequencing, using the same primers used for RT-qPCR (Table 2), was performed by the National Microbiology Laboratory in Winnipeg, Manitoba, run by the Public Health Agency of Canada.

Following sequencing, top matches of the consensus sequences for each sample were found using the BLAST tool from NCBI (Altschul *et al.* 1990; Sayers *et al.* 2025).

Virus phylogenetics

Both melt curve temperatures and sequences were relatively variable for SBV, 10 samples were successfully sequenced, and SBV PCR product sequences were relatively long at about 300bp, compared to the shorter sequences obtained from the other virus products (less than 300bp). Because of this, a phylogenetic tree was constructed including sequences uploaded to NCBI's GenBank to situate our samples (Sayers *et al.* 2025). First, all our SBV PCR product sequences were aligned using ClustalW in MEGA12 (v12.1.1) (Kumar *et al.* 2024), and any overhanging base pairs were removed so all sequences were the same length. Then, all sequences identified as *Iflavirus sacbroodi* that included a collection location, year, host species, and were longer than 290bp were downloaded from GenBank. These sequences were then aligned in batches to our SBV sequences in MEGA12 (Kumar *et al.* 2024) using ClustalW, both testing the forward and reverse complement directions, and any partial sequences that did not align with our sequences were excluded, as they mapped to different sections of the genome. All sequences that aligned with our samples were then cut so only the section of interest that corresponded to our sequences was retained. A phylogenetic tree was constructed with this alignment of 161 sequences using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993) of nucleotide substitutions in MEGA12 (Kumar *et al.* 2024). 1000 bootstrap replicates were then performed, and confidence values were displayed for nodes with values greater than 50% (Felsenstein 1985). The tree was then exported and visualized in FigTree (Rambaut 2018), and clades containing sequences from the same country and host species, or genus in the case of *Apis*, were collapsed with one representative sequence from the clade displayed.

A similar protocol was applied to the other four viruses, but sequences from GenBank were not included in their phylogenies. Sequences were aligned and a phylogeny was constructed

as described above, with the exception of IAPV. For IAPV, the phylogeny was instead inferred using the Neighbor-Joining method (Saitou and Nei 1987), and the evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.* 2004) because only three sequences were included in the analysis and at least 4 are required for the use of the Maximum Likelihood method in MEGA12. For trees with clades present, 1000 bootstrap replicates were performed and confidence values were displayed for all clades (Felsenstein 1985). Trees were exported and visualized in FigTree (Rambaut 2018). For all five viruses, the mean sequence divergence was calculated from each alignment of virus sequences, containing only sequences from this study. This was done by averaging the number of base substitutions between all sequence pairs, calculated using the Maximum Composite Likelihood method in MEGA12 (Tamura *et al.* 2004; Kumar *et al.* 2024).

RESULTS

Landscape overview and wild bee diversity

We selected 20 sites total for this study, ten of which were located on canola fields and ten of which were categorized as “non-agricultural”. This was done to ensure there was a range of agricultural intensities within field sites. Our field sites situated on canola fields had between 45.3% and 98.7% of land in the 1km radius surrounding each site designated as agricultural land use, while “non-agricultural” sites had between 0% and 44.8% of land designated as agricultural (Table 4).

Table 4: General site overview of land use, honey bee density, and wild bee metrics from transect data. The percent of each broad land use type present in the 1km radius around each site is listed. Only the percent of agricultural land use was used as a fixed effect in models. Land use types were designated as follows: urban (exposed land, urban/developed); semi-natural (water, shrubland, wetland, grassland, forests); agriculture (all crops, pasture/forages, too wet to be seeded). The measure of mean honey bees per transect is calculated as the average number of honey bees caught per transect across all six transects performed over the course of the summer at a site. Wild bee diversity is the Shannon diversity, and all wild bee metrics are calculated from the sum of wild bees caught across all six transects performed at a site.

Site type	Site	% Land use			Mean honey bees/ transect	Wild bee metrics (all transects)		
		Agriculture	Semi-natural	Urban		Diversity (<i>H</i>)	Species richness	Mean wild bees/ transect
Non-agricultural	FW	0.32	30.11	69.57	2.67	2.33	15	6.67
	TG	12.12	81.65	6.23	1.33	2.60	19	7.83
	SW	44.55	18.00	37.46	5.33	1.90	11	5.33
	LS	29.67	64.06	6.27	4.33	2.87	25	9.17
	BP	44.85	41.54	13.61	2.67	1.69	7	3.00
	LR	0.54	96.16	3.30	1.83	2.40	13	3.50
	SM	19.30	23.69	57.01	0.00	2.83	21	8.17
	MS	1.61	91.44	6.95	0.00	2.90	22	7.67
	RR	0.52	92.89	6.59	0.00	2.37	16	5.50
	SA	0.00	96.16	3.84	0.00	2.81	21	6.50
Agricultural (canola)	PV	91.42	1.23	7.35	3.17	1.33	4	0.83
	DW	93.17	1.64	5.20	2.33	1.39	5	1.83
	IN	88.15	9.87	1.98	10.50	1.93	8	2.17
	IS	87.45	10.38	2.18	10.83	2.12	11	4.00
	DM	58.05	8.22	33.73	3.83	1.40	5	3.83
	BN	95.67	0.54	3.78	0.00	1.30	5	2.33
	CM	45.25	23.59	31.16	7.00	1.75	8	2.67
	RT	98.74	1.20	0.06	4.17	1.56	5	1.00
	GL	95.53	1.35	3.13	1.33	1.28	4	1.17
	PS	91.49	1.23	7.27	1.67	1.56	5	1.00

At “agricultural” sites, the total number of flowering species over the course of the summer was between 10 and 40 species, and at “non-agricultural” sites it was between 11 and 52 species. All sites were located in the prairie ecozone except for the sites in Mars Sandhills (MS) and Richer (RR), which are located in the boreal plains ecozone, and Sandilands (SA), which

boarders the boreal plains and boreal shield ecozones. In total, 95 different wild bee species were collected in transect measurements. When looking at bees sampled in transects across the entire sampling period, wild bee species richness at “agricultural” sites varied between 4 and 11 species and Shannon diversity was between 1.28 and 2.12 (Table 4). At “non-agricultural” sites, wild bee species richness varied between 7 and 25 species and Shannon diversity was between 1.69 and 2.90 (Table 4).

When modeling wild bee diversity, the top model had only agricultural intensity (measured as % agriculture) as an explanatory variable (Table 5). In the global model, moderate collinearity was caused by the inclusion of SBV abundance and agricultural intensity in the same model (as these were found to be associated in models described later), but only agricultural intensity was included in the top model. Despite this, there were no detected collinearity issues in any models included in the model average, even when agriculture and SBV abundance were in the same model. The model with only SBV abundance as an explanatory variable performed considerably worse ($\Delta AICc = 12.9$) than the top model with only agricultural intensity, so agriculture appears to be the better predictor of wild bee diversity.

Table 5: Model selection results for models explaining wild bee diversity. The function MuMIn::dredge was applied to the global model. The global model included wild bee diversity as a response variable, and fixed effects of honey bee virus abundance, honey bee density, percent agriculture, flowering plant species richness, and percent floral cover. Only models with a $\Delta\text{AICc} < 2$ were included in the table and in the model average. Differences in values from the highest ranked model (ΔAICc), the number of parameters in each model (K), Akaike weights (w_i), and adjusted R squared values are included in the table. The null model is included for reference but was not included in the model average.

Parameters	K	AICc	ΔAICc	w_i	Adj R^2
% Agriculture	3	18.50	0.000	0.22	0.668
% Agriculture + Floral abundance	4	18.56	0.058	0.21	0.699
% Agriculture + Floral abundance + HB density	5	18.86	0.356	0.18	0.729
% Agriculture + Floral abundance + HB SBV abundance	5	18.91	0.408	0.18	0.728
% Agriculture + HB SBV abundance	4	20.02	1.516	0.10	0.676
% Agriculture + HB density	4	20.11	1.609	0.10	0.674
Null	2	38.82	20.32	NA	0

In the model average including all models with a ΔAICc of less than 2, again agricultural intensity was the only significant effect and had a negative effect on wild bee diversity (Table 6).

The effect of agricultural intensity in the top model is plotted in Figure 3.

Table 6: Model average results for parameters predicting wild bee diversity. Coefficient (β value), adjusted standard error (SE), z value, and p-value of each variable included in the model average explaining wild bee diversity. Models included in the model average were those with a $\Delta\text{AICc} < 2$ following the use of “dredge” on the global model, and the model average was calculated using the function “model.avg”. All values are reported from the full model average.

Parameter	β	Adjusted SE	z value	p-value
% Agriculture	-0.010	0.003	3.414	<0.001***
Floral abundance	-0.036	0.041	0.884	0.377
HB SBV abundance	-0.053	0.112	0.476	0.634
HB density	0.011	0.022	0.476	0.634
Intercept	2.662	0.156	17.03	<0.001***

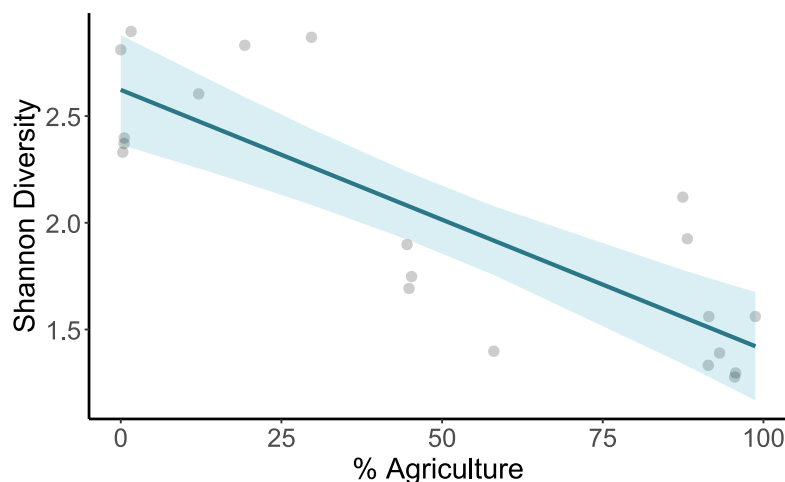


Figure 3: Adjusted predictions of % agriculture in the top model explaining wild bee diversity. Plots were created using the package “ggeffects” (Lüdtke 2018). The light blue area represents the 95% confidence interval.

Data overview of viruses in bees

A total of 425 honey bees and 588 wild bees from 63 species, across 21 genera and 5 families, were tested for black queen cell virus (BQCV), sacbrood virus (SBV), deformed wing virus A (DWV-A) and B (DWV-B), and Israeli acute paralysis virus (IAPV). Excluding honey bees, 42 different species of bees were detected with BQCV, 28 were detected with SBV, 20 were detected with DWV-B, 7 were detected with DWV-A, and 2 were detected with IAPV (Table 7).

Table 7: Virus detections by bee species. Detections of BQCV, SBV, DWV-B, DWV-A and IAPV by species, organized by family. The number of individuals tested per species is included. Species where some identifications were less certain but still plausible are denoted by “c.f.”.

Species	# of individuals tested	Virus				
		BQCV	SBV	DWV-B	DWV-A	IAPV
<i>Apis mellifera</i> Linnaeus, 1758	425	+	+	+	+	+
Apidae (sans <i>Apis</i>)						
<i>Anthophora terminalis</i> Cresson, 1869	5	+	+	-	-	-
<i>Bombus bimaculatus</i> Cresson, 1863	11	+	+	+	-	-
<i>Bombus borealis</i> Kirby, 1837	36	+	+	+	+	-
<i>Bombus griseocollis</i> (De Geer, 1773)	9	+	+	+	-	-
<i>Bombus melanopygus</i> Nylander, 1848	2	+	-	-	-	-
<i>Bombus nevadensis</i> Cresson, 1874	1	+	-	-	-	-
<i>Bombus rufocinctus</i> Cresson, 1863	72	+	+	+	-	-
<i>Bombus sandersoni</i> Franklin, 1913	19	+	+	+	-	-
<i>Bombus ternarius</i> Say, 1837	41	+	+	+	-	-
<i>Bombus terricola</i> Kirby, 1837	2	+	+	-	-	-
<i>Bombus vagans</i> Smith, 1854	31	+	+	-	+	-
<i>Ceratina calcarata</i> Robertson, 1900	4	+	-	-	-	-
<i>Ceratina dupla</i> Say, 1837	2	-	-	-	-	-
<i>Ceratina mikmaqi</i> Rehan and Sheffield, 2011	34	-	-	+	-	-
<i>Epeolus minimus</i> (Robertson, 1902)	1	-	+	-	-	-
<i>Melissodes confusus</i> Cresson, 1878	5	+	+	-	-	-
<i>Nomada c.f. composita</i> Mitchell, 1962	5	+	+	-	-	-
Megachilidae						
<i>Heriades carinata</i> Cresson, 1864	48	+	+	+	-	-
<i>Heriades variolosa</i> (Cresson, 1872)	4	-	-	+	-	-
<i>Hoplitis pilosifrons</i> (Cresson, 1864)	3	+	+	-	-	-
<i>Hoplitis producta</i> (Cresson, 1864)	8	+	+	-	-	-
<i>Megachile gemula</i> Cresson. 1878	1	-	-	-	-	-
<i>Megachile inermis</i> Provancher, 1888	12	+	+	-	-	+
<i>Megachile latimanus</i> Say, 1823	5	+	+	-	-	-
<i>Megachile melanophaea</i> Smith, 1853	9	-	-	-	-	-
<i>Megachile perihirta</i> Cockerell, 1898	1	-	-	-	-	-
<i>Megachile relativa</i> Cresson, 1878	15	+	+	-	-	-
<i>Megachile rotundata</i> (Fabricius, 1787)	1	-	-	+	-	-
<i>Osmia atriventris</i> Cresson, 1864	4	-	-	-	-	-

<i>Osmia proxima</i> Cresson, 1864	1	-	-	-	-	-
<i>Stelis coarctatus</i> Crawford, 1916	2	+	-	-	-	-
<i>Stelis lateralis</i> Cresson, 1864	7	+	+	-	-	-
Andrenidae						
<i>Andrena alleghaniensis</i> Viereck, 1907	4	+	+	-	-	-
<i>Andrena barbilabris</i> (Kirby, 1802)	2	+	+	+	-	-
<i>Andrena cyanophila</i> Cockerell, 1906	4	+	-	-	-	-
<i>Andrena c.f. miranda</i> Smith, 1879	5	+	+	+	-	-
<i>Andrena thaspii</i> Graenicher, 1903	15	+	+	-	+	+
<i>Andrena wilkella</i> (Kirby, 1802)	17	+	+	+	+	-
<i>Calliopsis andreniformis</i> Smith, 1853	5	+	-	-	-	-
<i>Protandrena perlaevis</i> (Cockerell, 1896)	1	-	-	-	-	-
Halictidae						
<i>Agapostemon sericeus</i> (Forster, 1771)	5	-	-	-	-	-
<i>Augochlorella aurata</i> (Smith, 1853)	15	-	-	+	-	-
<i>Dufourea marginata</i> (Cresson, 1878)	10	+	+	-	-	-
<i>Halictus confusus</i> Smith, 1853	2	+	+	+	+	-
<i>Halictus rubicundus</i> (Christ, 1791)	2	+	-	+	+	-
<i>Lasioglossum acuminatum</i> McGinley, 1986	1	-	-	-	-	-
<i>Lasioglossum albipenne</i> (Robertson, 1890)	12	+	-	-	-	-
<i>Lasioglossum leucozonium</i> (Schrank, 1781)	8	-	-	-	-	-
<i>Lasioglossum c.f. novascotiae</i> (Mitchell, 1960)	5	+	-	-	-	-
<i>Lasioglossum occidentale</i> (Crawford, 1902)	18	+	+	-	-	-
<i>Lasioglossum perpunctatum</i> (Ellis, 1913)	12	+	-	+	-	-
<i>Lasioglossum c.f. semicaeruleum</i> (Cockerell, 1895)	5	+	-	+	-	-
<i>Lasioglossum c.f. sheffieldi</i> Gibbs, 2010	1	-	-	-	-	-
<i>Lasioglossum viridatum</i> group	1	-	-	-	-	-
<i>Lasioglossum zonulus</i> (Smith, 1848)	1	+	-	-	-	-
Colletidae						
<i>Colletes hyalinus</i> Provancher, 1888	5	-	-	-	-	-
<i>Colletes kincaidii</i> Cockerell, 1898	1	-	-	-	-	-
<i>Hylaeus affinis</i> (Smith, 1853)	15	+	+	+	-	-
<i>Hylaeus annulatus</i> (Linnaeus, 1758)	4	+	-	-	-	-
<i>Hylaeus fedorica</i> (Cockerell, 1909)	2	-	-	+	+	-
<i>Hylaeus c.f. gaigei</i> (Cockerell, 1916)	1	-	-	-	-	-
<i>Hylaeus mesillae</i> (Cockerell, 1907)	1	+	-	-	-	-
<i>Hylaeus modestus</i> Say, 1837	7	+	-	-	-	-

Notably, all honey bees were detected with BQCV, while this was not the case for wild bees or for any other virus within honey bees or wild bees. The prevalence of each virus followed the same pattern in both honey bees and wild bees, with the highest prevalence seen in BQCV (100% in honey bees, 48.8% in wild bees), followed by SBV (69.6% in honey bees, 24.3% in wild bees), DWV-B (37.9% in honey bees, 5.95% in wild bees), DWV-A (13.9% in honey bees, 1.53% in wild bees) then IAPV (7.76% in honey bees, 0.51% in wild bees). For all viruses, prevalence was found to be significantly higher in honey bees when compared to wild bees (SBV: GLMM, $z = 12.54$, $p < 0.001$; DWV-B: GLMM, $z = 11.32$, $p < 0.001$; DWV-A: GLMM, $z = 6.73$, $p < 0.001$; IAPV: GLMM, $z = 4.58$, $p < 0.001$; BQCV: Fisher's exact test, $p < 0.001$). This pattern can be observed in (Figure 4A). Average intensity at the site level was higher in honey bees when compared to wild bees for BQCV (Mann-Whitney U Test, $U = 0$, $p < 0.001$, $n = 18, 19$), SBV (Welch's t Test, $t = -5.37$, $p < 0.001$, $df = 24.08$), and DWV-B (Mann-Whitney U Test, $U = 28$, $p = 0.002$, $n = 11, 16$), a trend that can be observed in Figure 4B. Sample sizes of DWV-A (9) and IAPV (3) in wild bees were insufficient to make intensity comparisons.

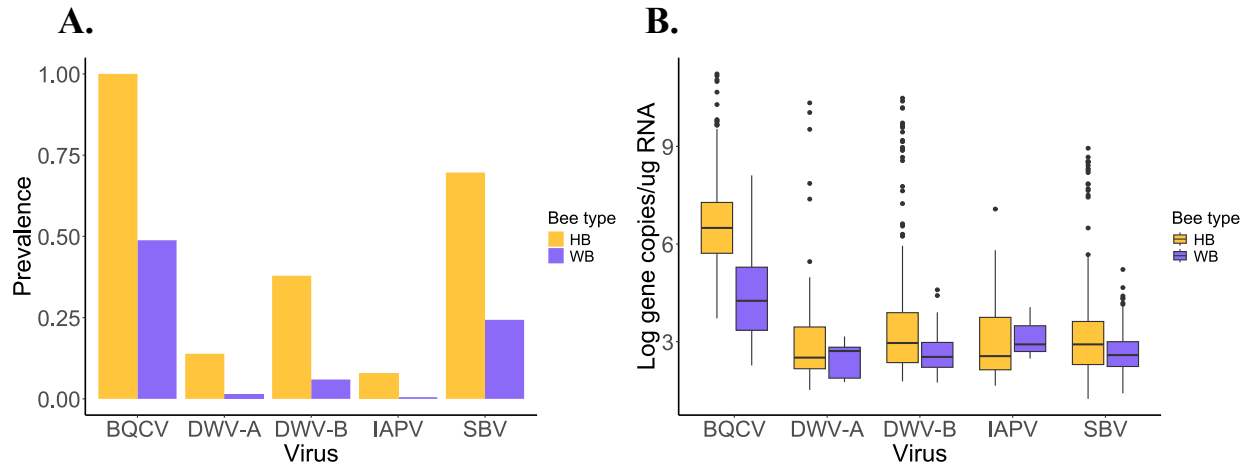


Figure 4: Prevalence (A) and intensity (B) of each virus (BQCV, SBV, DWV-B, DWV-A, IAPV) in honey bees (HB) and wild bees (WB). Prevalence is calculated as the proportion of bees with detectable virus. Intensity is calculated as the log(number of gene copies per ug of RNA +1) in bees with detectable virus.

Co-occurrences of viruses in both wild bee and honey bee samples were commonly observed (Figure 5). In 17.9% of honey bee samples only one virus was detected, 44.9% had two viruses, 28.5% had three, 7.5% had four, and in 1.2% of honey bee samples, all five tested viruses were detected. Because all honey bees were detected with BQCV, no samples had zero virus detections. In wild bees, coinfections were less common but still frequently observed, with less than half of samples (46.8%) being free of detectable virus, 28.2% having one detectable virus, 22.3% with two, 2.6% with three, and just one sample (0.2%), an *Andrena wilkella*, with four viruses detected.

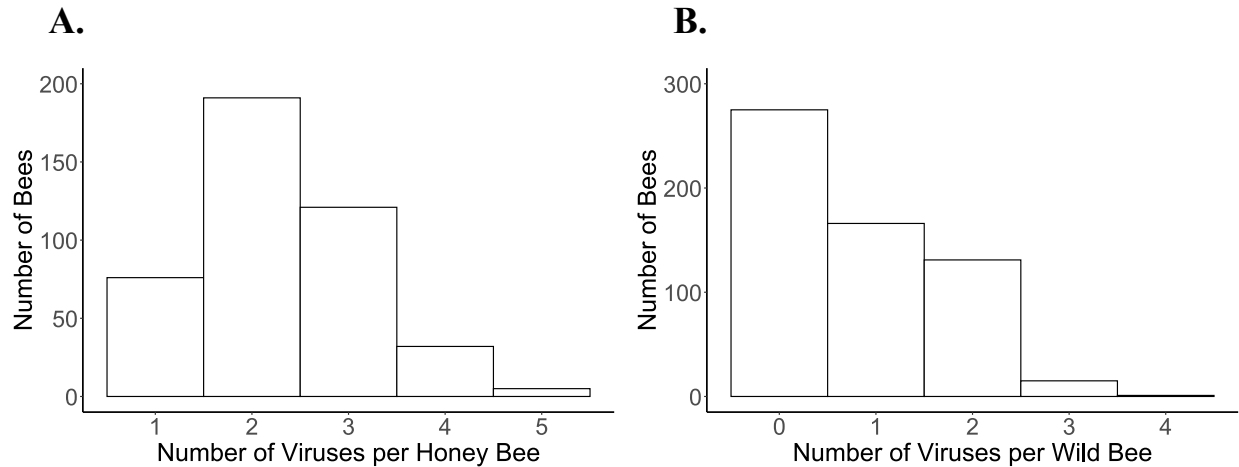


Figure 5: Virus co-occurrences in bees. The frequency of detecting each number of viruses in honey bees (A) and wild bees (B).

Modeling virus presence in wild bees

Modeling BQCV Presence in Wild Bees

The top model explaining BQCV presence in wild bees included the explanatory variables of honey bee BQCV abundance, percent agriculture, phylogenetic distance from honey bees and Julian date (Table 8). All other models with a $\Delta AICc$ from the top model of less than 2 included these four fixed effects in their models. The top model had a marginal R^2 value of 0.38, a conditional R^2 value of 0.66 (Table 8), and the random effects of species and site had variances of 1.81 (± 1.34) and 0.93 (± 0.97) respectively.

Table 8: Model selection results of models explaining BQCV presence in wild bees. The function MuMIn::dredge was applied to the global model. The global model included presence/absence of BQCV in each wild bee as a response variable, fixed effects of honey bee BQCV abundance, honey bee density, percent agriculture, phylogenetic distance from honey bees, Julian date, sex, sociality, flowering plant species richness, percent floral cover, and random effects of species and site. Only models with a $\Delta AICc < 2$ were included in the table and in the model average. Differences in values from the highest ranked model ($\Delta AICc$), the number of parameters in each model (K), Akaike weights (w_i), marginal R squared (without random effects), and conditional R squared (with random effects) values are included. Akaike weights (w_i) were taken from the model average, not the full table generated by “dredge”. A model containing only fixed effects and the null model are included for reference but were not included in the model average. All models except the null model include species and site as random effects.

Parameters	K	AICc	$\Delta AICc$	w_i	R^2_m	R^2_c
HB BQCV abundance + % Agriculture + Phylogenetic distance from HB + Date	7	571.6	0.000	0.34	0.381	0.662
HB BQCV abundance + % Agriculture + Phylogenetic distance from HB + Date + HB density	8	572.7	1.127	0.19	0.386	0.657
HB BQCV abundance + % Agriculture + Phylogenetic distance from HB + Date + Sex	8	572.9	1.364	0.17	0.379	0.667
HB BQCV abundance + % Agriculture + Phylogenetic distance from HB + Date + Floral abundance	8	573.2	1.592	0.15	0.380	0.666
HB BQCV abundance + % Agriculture + Phylogenetic distance from HB + Date + Flowering plant species richness	8	573.3	1.768	0.14	0.381	0.669
Random effects	3	610.0	38.46	NA	0	0.644
Null	1	816.8	245.3	NA	0	NA

The four fixed effects included in the top model were all significant predictors of wild bee BQCV presence when calculated from the full model average (Table 9). The marginal effects of each significant variable are plotted for the top model in Figure 7. Because variables were scaled prior to modeling, the sign of the β value is more informative than the value. Wild bees caught at sites with a greater abundance of BQCV in honey bees had a greater chance having detectable BQCV (Figure 7A). Additionally, wild bees more phylogenetically distant

from honey bees had a lower chance of having detectable BQCV, while those more closely related to honey bees were at a greater risk of being detected with BQCV (Figure 7C). Increasing agricultural intensity at sites also increased that chance of BQCV detections in wild bees at these sites (Figure 7E). It was also found that as the summer progressed, the chance of detecting BQCV in wild bees increased (Figure 7G). All other variables included in the global model were not significant.

Table 9: Model average results of parameters predicting BQCV presence in wild bees. Coefficient (β value), adjusted standard error (SE), z value, and p-value of each variable included in the model average explaining WB BQCV presence/absence. Models included in the model average were those with a $\Delta AICc < 2$ following the use of “dredge” on the global model, and the model average was calculated using the function “model.avg”. All values are reported from the full model average.

Parameter	β	Adjusted SE	z value	p-value
HB BQCV abundance	1.161	0.503	2.310	0.021*
HB density	0.0450	0.140	0.321	0.748
% Agriculture	1.401	0.486	2.884	0.004**
Phylogenetic distance from HB	-7.933	1.685	4.708	<0.001***
Date	4.943	1.611	3.068	0.002**
Sex – male	0.0536	0.196	0.273	0.785
Floral abundance	-0.0163	0.0725	0.225	0.822
Flowering species richness	0.0371	0.209	0.178	0.859
Intercept	0.576	2.380	0.242	0.809

Modeling SBV Presence in Wild Bees

The fixed effects included in the top model explaining SBV presence in wild bees, like BQCV, included honey bee BQCV abundance, percent agriculture, phylogenetic distance from honey bees, and Julian date, but it also included sociality (Table 10). All other models with a $\Delta AICc$ value of less than 2 also included these five variables. The marginal R^2 value of the top

model was 0.37, and the conditional R^2 value was 0.49 (Table 10). The variances in the top model for the random effects of species and site were 0.07 (± 0.26) and 0.57 (± 0.76) respectively.

Table 10: Model selection results of models explaining SBV presence in wild bees. The function MuMIn::dredge was applied to the global model. The global model included presence/absence of SBV in each wild bee as a response variable, fixed effects of honey bee SBV abundance, honey bee density, percent agriculture, phylogenetic distance from honey bees, Julian date, sex, sociality, flowering plant species richness, percent floral cover, and random effects of species and site. Only models with a $\Delta AICc < 2$ were included in the table and in the model average. Differences in values from the highest ranked model ($\Delta AICc$), the number of parameters in each model (K), Akaike weights (w_i), marginal R squared, and conditional R squared values are included. Akaike weights (w_i) were taken from the model average, not the full table generated by “dredge”. A model containing only fixed effects and the null model are included for reference but were not included in the model average. All models except the null model include species and site as random effects.

Parameters	K	AICc	$\Delta AICc$	w_i	R^2_m	R^2_c
HB SBV abundance + % Agriculture + Phylogenetic distance from HB + Date + Sociality	9	504.3	0.000	0.44	0.373	0.494
HB SBV abundance + % Agriculture + Phylogenetic distance from HB + Date + Sociality + Flowering species richness	10	506.0	1.650	0.19	0.373	0.499
HB SBV abundance + % Agriculture + Phylogenetic distance from HB + Date + Sociality + HB density	10	506.0	1.687	0.19	0.373	0.493
HB SBV abundance + % Agriculture + Phylogenetic distance from HB + Date + Sociality + Sex	10	506.1	1.753	0.18	0.373	0.493
Random effects	3	554.6	50.27	NA	0	0.475
Null	1	654.4	150.1	NA	0	NA

The five fixed effects included in the top model were also significant when evaluated in the model average (Table 11). Results for the four fixed effects significant for predicting BQCV presence in wild bees were similar for SBV (Figure 7). Wild bees caught from sites with increased honey bee SBV abundance also had a greater chance of having detectable SBV (Figure 7B). Increased agricultural intensity (Figure 7F) and later dates in the summer (Figure 7H) also

both increased the chance of detecting SBV in wild bees. Wild bees more phylogenetically distant from honey bees also again were less likely to be detected with SBV compared to those more closely related (Figure 7D). Unique to SBV was the effect of kleptoparasitism, as kleptoparasitic bees had a greater chance of having detectable SBV compared to eusocial or solitary bees (Figure 6). All other fixed effects explaining SBV presence were not significant.

Table 11: Model average results of parameters predicting SBV presence in wild bees. Coefficient (β value), adjusted standard error (SE), z value, and p-value of each variable included in the model average explaining WB SBV presence/absence. Models included in the model average were those with a $\Delta AICc < 2$ following the use of “dredge” on the global model, and the model average was calculated using the function “model.avg”. All values are reported from the full model average.

Parameter	β	Adjusted SE	z value	p-value
HB SBV abundance	0.592	0.281	2.108	0.035*
HB density	0.129	0.208	0.619	0.536
% Agriculture	0.890	0.402	2.211	0.027*
Phylogenetic distance from HB	-6.517	1.550	4.203	>0.001***
Date	5.248	1.382	3.796	>0.001***
Sociality – eusocial	-0.127	0.568	0.223	0.823
Sociality – parasitic	1.939	0.713	2.719	0.007**
Sex – male	-0.211	0.377	0.560	0.576
Flowering species richness	0.279	0.436	0.640	0.522
Intercept	-1.789	2.367	0.756	0.450

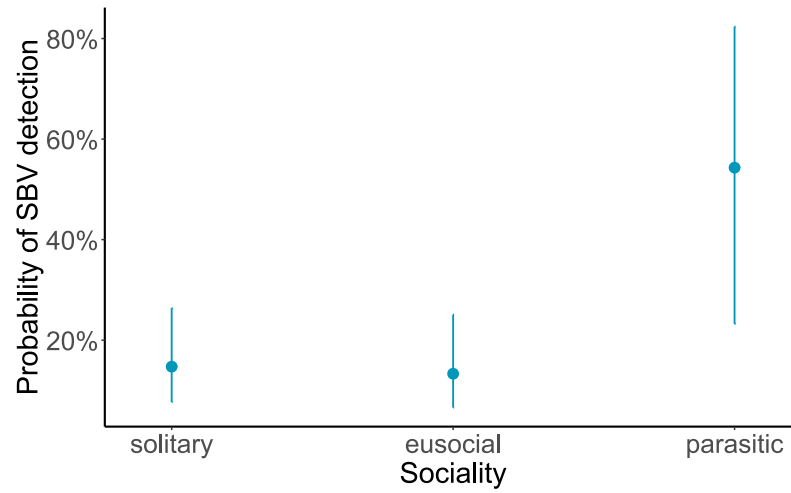


Figure 6: Adjusted predictions of sociality in the top model explaining SBV presence in wild bees. Plots were created using the package “ggeffects” (Lüdtke 2018). The blue bars represent 95% confidence intervals.

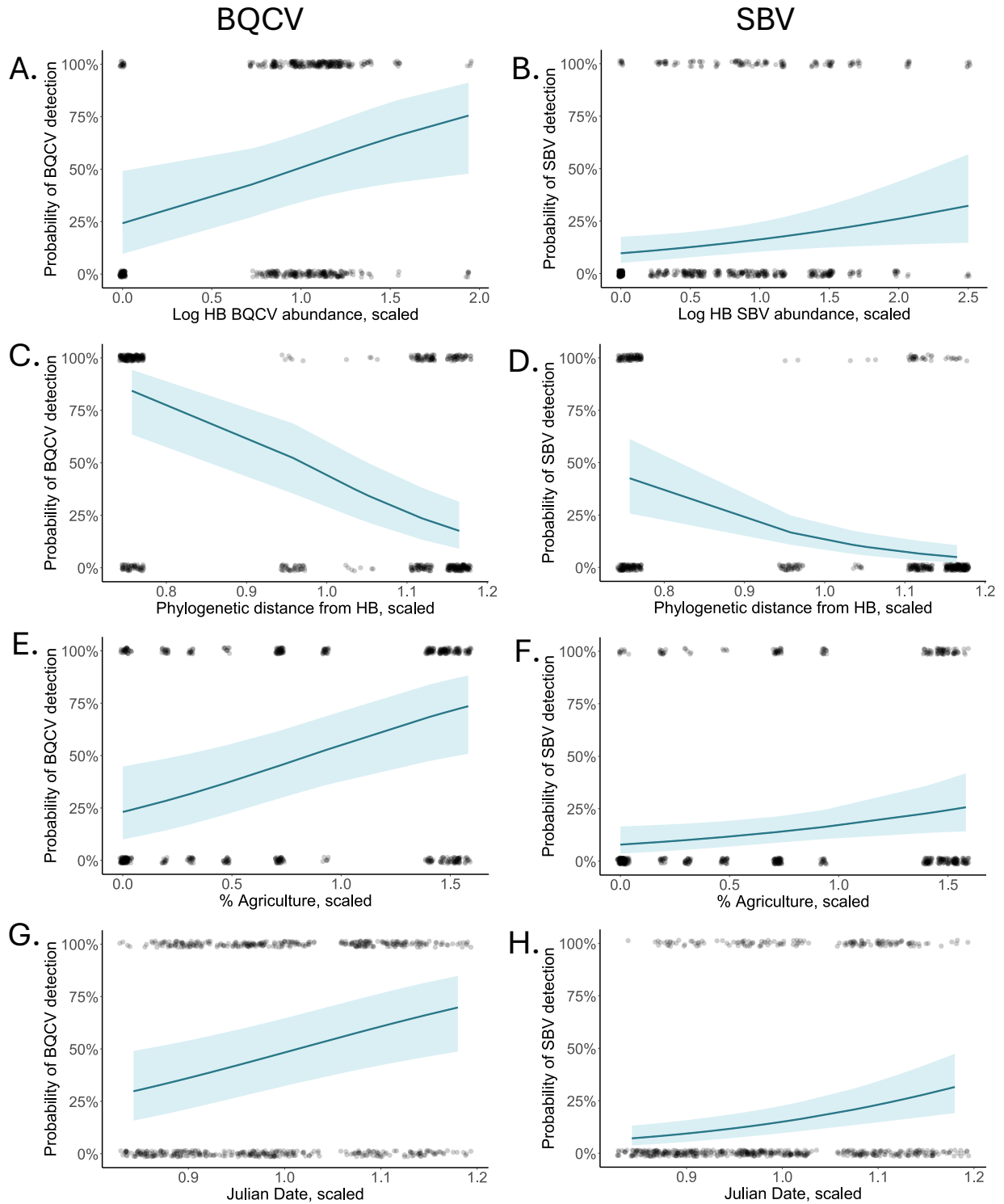


Figure 7: Adjusted predictions for the fixed effects of virus abundance in honey bees (A, B), phylogenetic distance (C, D), agriculture (E, F), and date G, H) in the top models explaining BQCV (A, C, E, G) and SBV (B, D, F, H) presence in wild bees. Plots were created the package “ggeffects” (Lüdtke 2018). The light blue areas represent 95% confidence intervals. A jitter of 0.015 was included for readability.

Modeling DWV-B Presence in Wild Bees

Only two fixed effects were present in the top model explaining DWV-B presence in wild bees. Like BQCV and SBV, honey bee DWV-B abundance was included, but floral abundance was also in the top model (Table 12). Both variables were also in all models with a ΔAICc of less than 2. The marginal R^2 value of this top model was 0.07, while the conditional R^2 value was 0.54 (Table 12). In the top model, the random effect of species had a variance of 1.16 (± 1.08) and the random effect of site had a variance of 2.19 (± 1.48). Sociality was removed from the global model because there were no kleptoparasitic bees with detectable DWV-B.

Table 12: Model selection results of models explaining DWV-B presence in wild bees. The function MuMIn::dredge was applied to the global model. The global model included presence/absence of DWV-B in each wild bee as a response variable, fixed effects of honey bee DWV-B abundance, honey bee density, percent agriculture, phylogenetic distance from honey bees, Julian date, sex, flowering plant species richness, percent floral cover, and random effects of species and site. Only models with a $\Delta AICc < 2$ were included in the table and in the model average. Differences in values from the highest ranked model ($\Delta AICc$), the number of parameters in each model (K), Akaike weights (w_i), marginal R squared, and conditional R squared values are included. All models except the null model include species and site as random effects. Akaike weights (w_i) were taken from the model average, not the full table generated by “dredge”. A model containing only fixed effects and the null model are included for reference but were not included in the model average.

Model	K	AICc	$\Delta AICc$	w_i	R^2_m	R^2_c
HB DWV-B abundance + Floral abundance	5	230.9	0.000	0.19	0.067	0.538
HB DWV-B abundance + Floral abundance + Date	6	231.3	0.388	0.16	0.079	0.532
HB DWV-B abundance + Floral abundance + % Agriculture	6	232.0	1.071	0.11	0.103	0.556
HB DWV-B abundance + Floral abundance + Sex	6	232.1	1.131	0.11	0.078	0.522
HB DWV-B abundance + Floral abundance + Date + % Agriculture	7	232.3	1.373	0.10	0.119	0.553
HB DWV-B abundance + Floral abundance + Date + HB density	7	232.5	1.551	0.09	0.103	0.535
HB DWV-B abundance + Floral abundance + Date + Sex	7	232.5	1.569	0.09	0.091	0.515
HB DWV-B abundance + Floral abundance + HB density	6	232.5	1.569	0.09	0.081	0.536
HB DWV-B abundance + Floral abundance + Flowering species richness	6	232.9	1.999	0.07	0.068	0.538
Random effects	3	238.6	7.688	NA	0	0.519
Null	1	267.4	36.46	NA	0	NA

Only two variables were significant in predicting DWV-B presence: honey bee DWV-B abundance at the site, and floral abundance (Table 13). Similar to the results for BQCV and SBV, the chance of detecting DWV-B in wild bees increased with increased honey bee DWV-B abundance at the site (Figure 8A). Counter to the previous viruses, floral coverage was

significant, and as floral abundance increased so did the chance of detecting DWV-B in wild bees (Figure 8B). No other fixed effects were significant predictors.

Table 13: Model average results of parameters predicting DWV-B presence in wild bees. Coefficient (β value), adjusted standard error (SE), z value, and p-value of each variable included in the model average explaining WB DWV-B presence/absence. Models included in the model average were those with a $\Delta AICc < 2$ following the use of “dredge” on the global model, and the model average was calculated using the function “model.avg”. All values are reported from the full model average.

Parameter	β	Adjusted SE	z value	p-value
HB DWV-B abundance	0.667	0.299	2.232	0.026*
HB density	0.055	0.201	0.275	0.783
% Agriculture	0.153	0.454	0.338	0.736
Date	-1.345	2.204	0.610	0.542
Sex – male	-0.131	0.420	0.312	0.755
Floral abundance	0.412	0.204	2.016	0.044*
Flowering species richness	-0.010	0.194	0.054	0.957
Intercept	-3.514	2.306	1.524	0.128

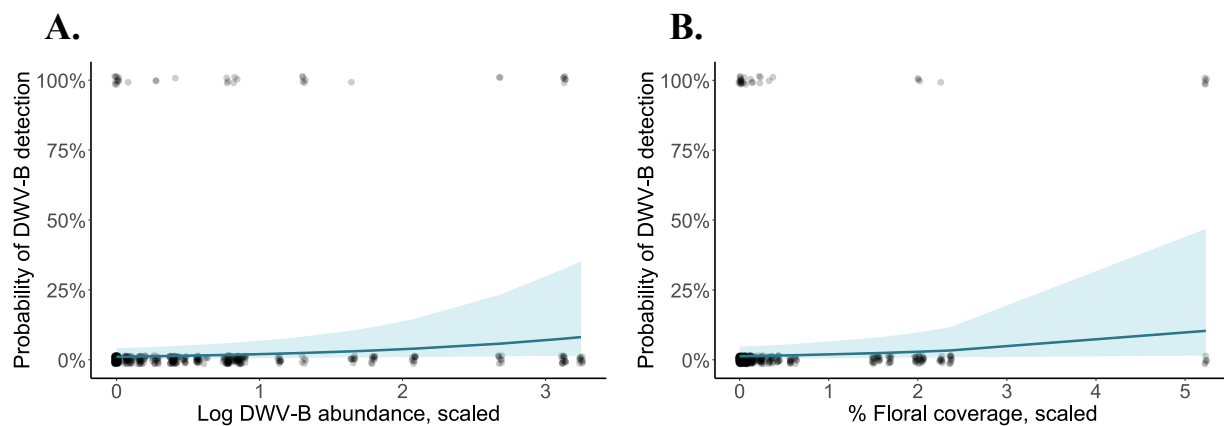


Figure 8: Adjusted predictions for the fixed effects of virus abundance in honey bees (A) and floral abundance (B) in the top model explaining DWV-B presence in wild bees. Plots were created using the package “ggeffects” (Lüdtke 2018). The light blue areas represent 95% confidence intervals. A jitter of 0.015 was included for readability.

Virus sequencing results

When performing RT-qPCR, a range of peak melt curve temperatures were observed for all five viruses. BQCV was the least variable virus with consistent melt curves, and the melt temperatures observed in samples, between 81.0°C and 81.5°C, were also seen in the gBlock™ standards. SBV had melt curve temperatures ranging from 81.5°C to 82.5°C in honey bees, with some low-level detections in wild bees falling outside this range, and appeared to be the most consistently variable virus in terms of melt curve temperatures within the same site. DWV-B had a melt curve temperature range of 78°C to 79.5°C, DWV-A had a range of 80.0°C to 81.5°C, and IAPV had arrange of 81.5°C to 82.5°C. To verify that all these samples still belong to the virus tested, sequencing was performed on select PCR product samples.

Due to high quantification threshold (Cq) values, not every peak melt curve temperature was able to have virus sequenced for both honey bees and wild bees, but this was done when it was possible. All melt temperatures other than IAPV at 82.5°C, due to low levels of detection and few samples, were tested with at least one sample. Additional samples for SBV with peak melt temperatures outside of the range found in honey bees were tested to confirm that these were off-target gene detections.

All samples that fell within the melt curve temperatures observed in honey bees matched the target virus when using the BLAST tool from NCBI (Table S3) (Altschul *et al.* 1990; Sayers *et al.* 2025). This verified that although there is sequence variation causing variation in the melt curves of viruses, these are still accurate detections. Additionally, for SBV detections outside of the melt temperature range observed in honey bees, sequences most closely matched genes found in related bee species, confirming they were off target detections (Table S3). In an off-target SBV sample from *Bombus borealis*, BP2WB0, an SBV sequence was also sequenced along with

the off-target gene (Table S3). In the 3 wells tested for this sample, the melt curve of one of the wells appeared to be skewed left by another sequence, which was likely the SBV sequence. Because a cutoff needed to be enforced to separate off-target detections from true virus detections, only samples with melt curves peak temperatures within the range detected in honey bees listed above were considered positive.

From these SBV PCR product sequences, a phylogeny was made including sequences publicly available on GenBank to situate our samples in the global phylogeny. The final phylogeny contained 161 sequences, including the 10 from our study, most of which were from *A. mellifera* and *Apis cerana* Fabricius, 1793, the Asian honey bee. Three of our sequences were from *A. mellifera*, while the other seven sequences were from various wild bee species. Our samples clustered with other samples found in North America, and sequences from our honey bees were interspersed with those from our wild bees, showing no evidence of clustering based on host species. Sequences tended to cluster based on broad geographic location, with some mixing between sequences in Oceania and Asia. Additionally, the gBlock™ used as a standard in our qPCR protocol has a sequence matching that of KC007374.1 from Vietnam (highlighted in yellow, Figure 9), which is relatively phylogenetically far from the North American clade containing our samples (highlighted in orange, Figure 9).

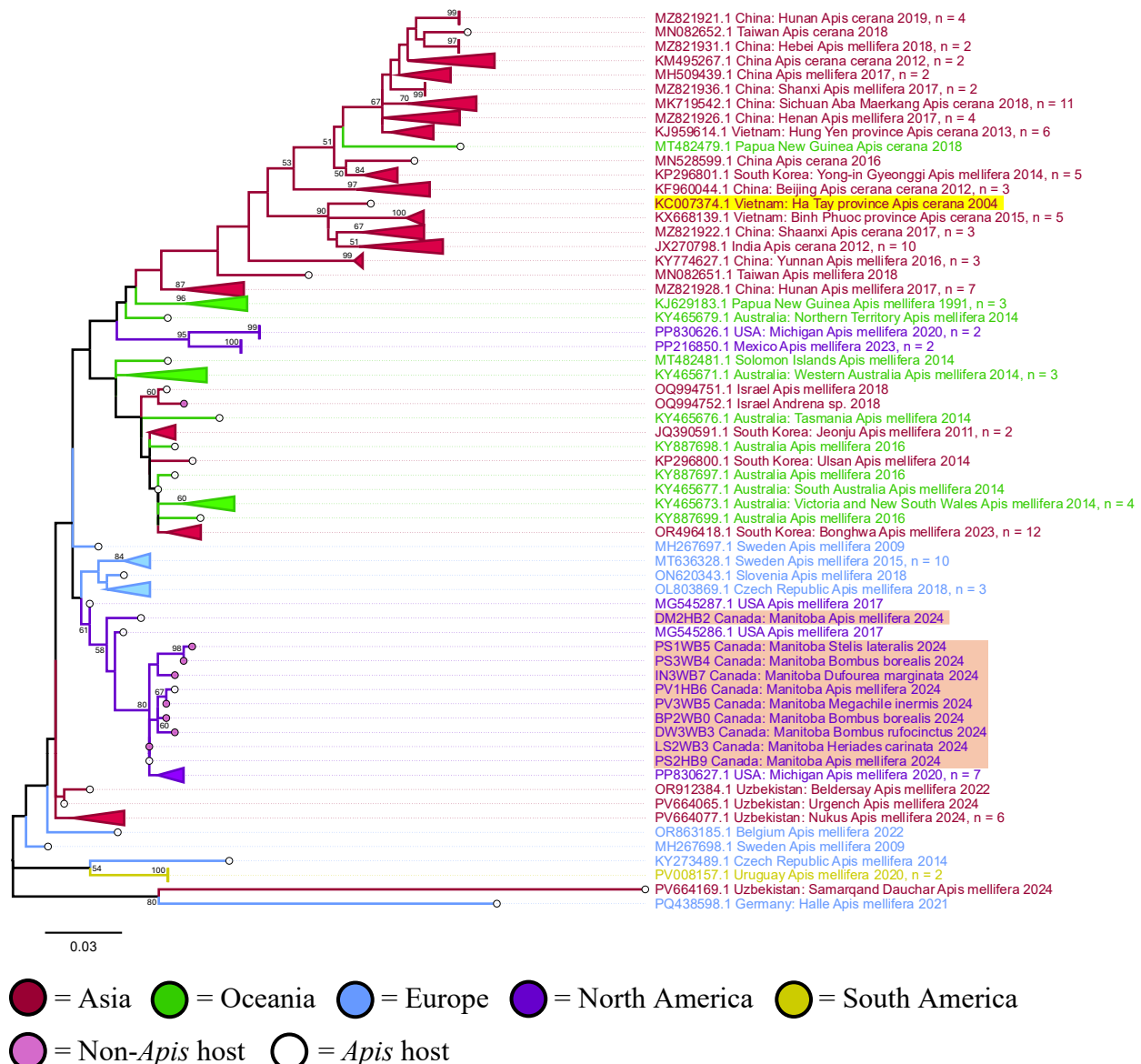


Figure 9: Evolutionary analysis of SBV. Analysis was performed using study samples and those available from GenBank aligning with the study samples, and was conducted in MEGA12 (Kumar *et al.* 2024) and visualized with FigTree (Rambaut 2018). The phylogeny was constructed using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993) of nucleotide substitutions using MEGA12 (Kumar *et al.* 2024). The displayed tree is the one with the highest log likelihood (-4322.87) and is unrooted. Bootstrap support (1000 replicates) is shown for nodes with values greater than 50% (Felsenstein 1985). The analysis comprised of 161 sequences and 306 nucleotide positions. Colours of nodes and labels are based on continent, and clades of the same country and host species, or genus if the host is within *Apis*, are collapsed. The number of sequences in the clade are specified, along with a representative sequence for the clade. Tips are coloured based on whether the sequence was from a non-*Apis* (pink) or *Apis* (white) host. Sequences highlighted in orange are from this study, and the sequence matching the SBV gBlock™ used is highlighted in yellow.

Phylogenetic trees were also constructed for the other four viruses to observe if there was any evidence of clustering based on host species, but only sequences from the study were included (Figure 10). Like with SBV, wild bee sequences were situated among sequences from *A. mellifera*, with no evidence of clustering. A phylogenetic tree including all DWV sequences was also constructed, confirming that each variant was phylogenetically distinct in the region sequenced (Figure S2).

All but two SBV samples had unique sequences, and the mean sequence divergence (d) between the 10 study samples was 0.018. The mean sequence divergence was similar all other viruses except BQCV, and were 0.015 for DWV-B, 0.019 for DWV-A, 0.024 for IAPV, and 0.0029 for BQCV. The lower sequence divergence in BQCV was expected because the sequenced BQCV samples were identical in sequence for three of the five samples which spanned 4 host families, and the two divergent sequences only varied by one base pair each from the 3 identical sequences. Similarly, the peak melt curve temperatures appeared more consistent for BQCV samples when compared to the other viruses.

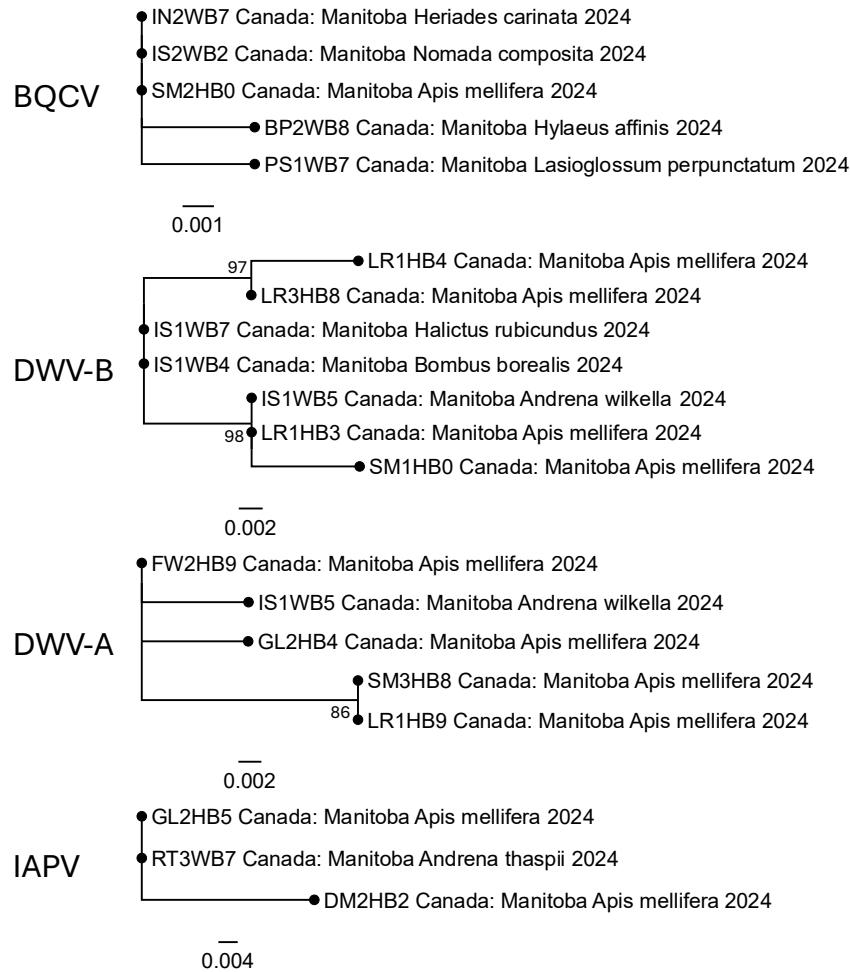


Figure 10: Evolutionary analysis of BQCV, DWV-B, DWV-A, and IAPV. Analysis was performed using study samples and was conducted in MEGA12 (Kumar *et al.* 2024) and visualized with FigTree (Rambaut 2018). For all viruses except IAPV, the phylogenies were constructed using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993) of nucleotide substitutions using MEGA12 (Kumar *et al.* 2024). The displayed trees are those with the highest log likelihoods. The IAPV phylogeny was constructed using the Neighbor-Joining method (Saitou and Nei 1987), and the evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.* 2004) and are in the units of the number of base substitutions per site. Bootstrap support (1000 replicates) is displayed for all clades present (Felsenstein 1985). The BQCV analysis comprised of 5 sequences and 280 nucleotide positions, the DWV-B analysis comprised of 7 sequences and 112 nucleotide positions, the DWV-A analysis comprised of 5 sequences and 109 nucleotide positions, and the IAPV analysis comprised of 3 sequences and 203 nucleotide positions. All trees are unrooted.

Internal controls for bees in RT-qPCR

The most successful gene tested as a reference gene, which was detected in all bees and showed a close alignment of melt curves, was S28, a ribosomal gene from *Bombus terrestris* (Figure 11) (Sankar *et al.* 2024). This was then used as an internal control for all bees. It was detected, with varying melt curve temperatures and levels of detection (but consistent within species), in all bees except *Colletes hyalinus*, although Cq values were high for *Colletes kincaidii* (Cq ~39), *Protandrena perlaevis* (Cq ~ 39), and *Anthophora terminalis* (Cq ~ 38). Once it was discovered that S28 could not be detected in *C. hyalinus*, of which there were five samples, three of the five plates with these samples used Opsin primers, for the long-wavelength rhodopsin gene (Radzevičiūtė *et al.* 2017), instead of S28 as a reference gene because these primers have been shown to successfully amplify Opsin in *Colletes* (Almeida and Danforth 2009). Opsin was detected for all *C. hyalinus*, verifying the integrity of these samples.

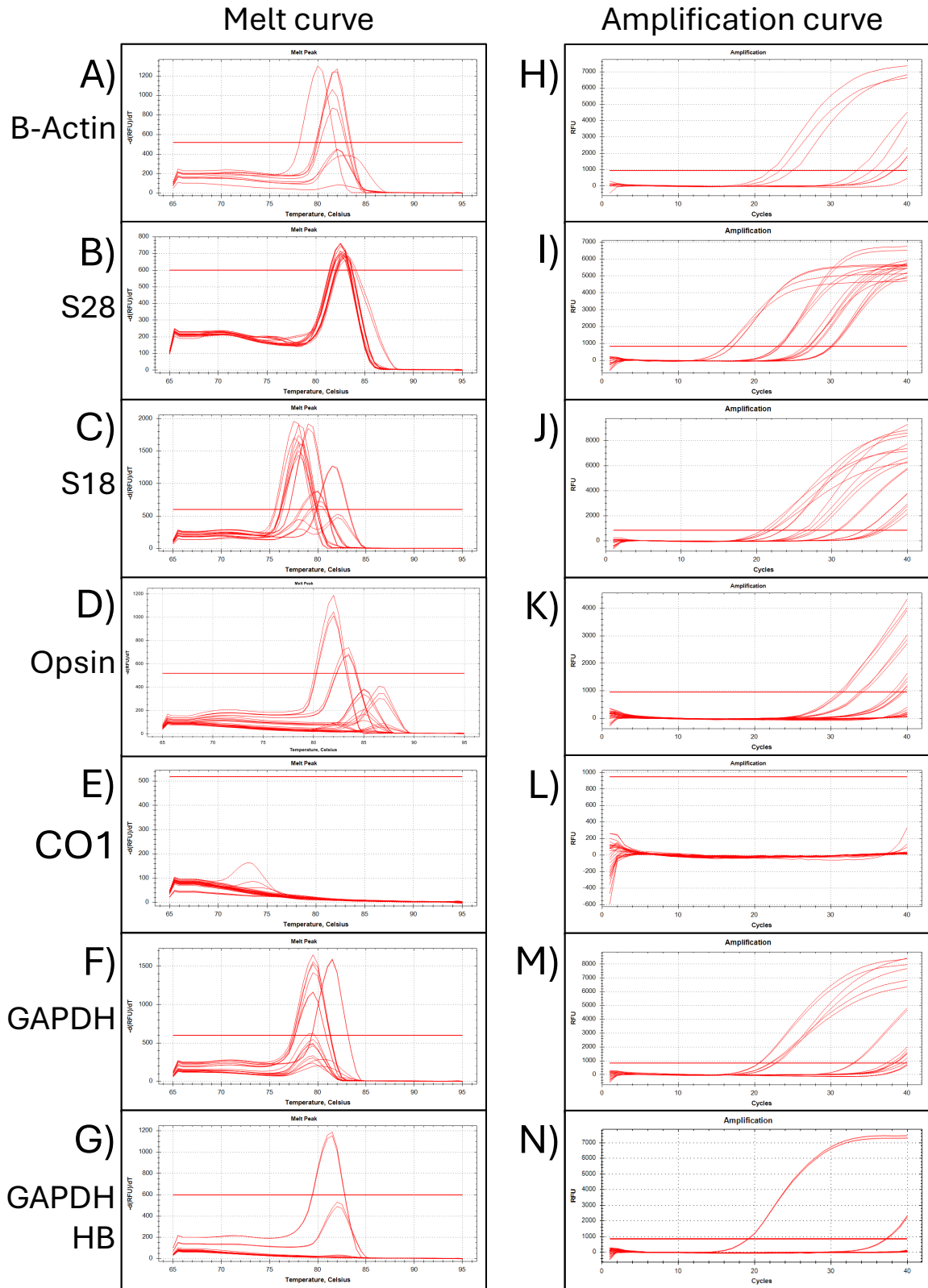


Figure 11: Melt curves (A-G) and amplification curves (H-N) for the 7 primer pairs tested as reference genes. Actin (A, H) was tested with single wells, Opsin (D, K) and CO1 (E, L) were tested in triplicate, and all other primers were tested in duplicate.

Data overview of viruses from pollen

A total of 56 pollen samples were tested from *Bombus* spp. with pollen loads categorized at medium or larger when they were removed. The prevalence of viruses followed a similar pattern to virus prevalence in bees, with BQCV being the most prevalent, followed by SBV, then DWV-B (Figure 12). There were no detections of DWV-A or IAPV in the tested pollen samples.

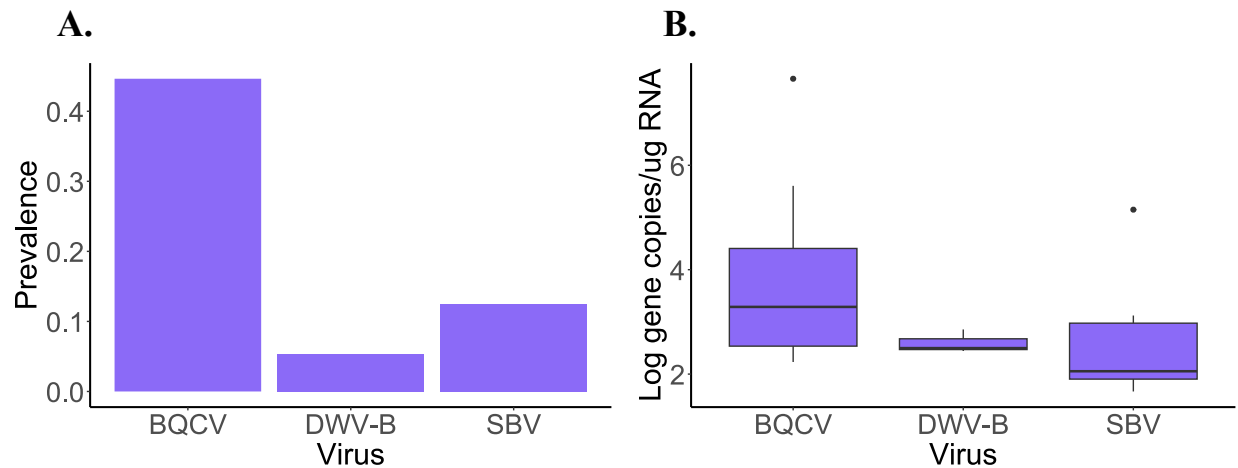


Figure 12: Prevalence (A) and intensity (B) of each detected virus (BQCV, SBV, DWV-B) in pollen collected from *Bombus* spp. Prevalence is calculated as the proportion of bees with detectable virus. Intensity is calculated as the log(number of gene copies per ug of RNA +1) in pollen samples with detectable virus.

Modeling virus presence in pollen

BQCV in Pollen

The results of model selection using the function “dredge” on the global model for BQCV in pollen placed the model containing only BQCV abundance from all bees collected at the site as a fixed effect as the top ranked model, followed by the model containing BQCV abundance and date (Table 14). These were the only two models with a delta AICc of less than 2, so they were the only models in the model average used to evaluate which predictors of BQCV

presence in pollen were significant (Table 14). The variance of site in the top model was 1.11 ($\pm$ SD 1.05) and 1.22 ($1.10 \pm$ SD) in the second model.

Table 14: Initial model selection results of models explaining BQCV presence in pollen. The function MuMIn::dredge was applied to the global model. The global model included presence/absence of BQCV in each pollen sample as a response variable, fixed effects of BQCV abundance of the wild and honey bees, percent agriculture, Julian date, and site as a random effect. Only models with a $\Delta AICc < 2$ were included in the table and in the model average. Differences in values from the highest ranked model ($\Delta AICc$), the number of parameters in each model (K), Akaike weights (w_i), and marginal and conditional R squared values are included. Akaike weights (w_i) were taken from the model average, not the full table generated by “dredge”.

Parameters	K	AICc	$\Delta AICc$	w_i	R^2_m	R^2_c
WB and HB BQCV abundance	3	58.78	0	0.60	0.400	0.551
WB and HB BQCV abundance + Date	4	59.57	0.79	0.40	0.468	0.612

In the model average, BQCV abundance was significant and positive, indicating a positive relationship between the chance of BQCV detection in pollen and BQCV abundance at the site, and it was the only significant fixed effect (Table 15).

Table 15: Model average results of parameters predicting BQCV presence in pollen. Coefficient (β value), standard error, z value, and corresponding p-value of each variable within the full model average explaining BQCV presence in pollen. A model average was created with “model.avg” using models with a $\Delta AICc < 2$ after using “dredge” on the global model.

Parameter	β	Adjusted SE	z value	p-value
WB and HB BQCV abundance	4.561	1.754	2.601	0.009**
Date	2.553	4.694	0.544	0.587
Intercept	-7.217	5.462	1.321	0.186

Because agricultural intensity and date were not significant, they were not included in the candidate model set. The best performing model was the model with only BQCV gene copies in the bee that pollen was collected from as a fixed effect (Table 16). The next best performing model was the model with BQCV abundance as a fixed effect, and it had a $\Delta AICc$ of 14.10 from

the top model (Table 16). Both models performed better than the random effect and null models.

The variance of site was 1.99 ($\pm$ SD 1.41) in the top model.

Table 16: Secondary model selection results of models explaining BQCV presence in pollen. The function MuMIn::model.sel was applied to the candidate model set to compare the predictive power of BQCV in the bee that pollen was collected from to BQCV abundance in all collected bees on pollen BQCV presence. Differences in values from the highest ranked model (Δ AICc), the number of parameters in each model (K), Akaike weights (w_i), and marginal and conditional R squared values are included. All models except the null model include site as random effect.

Parameters	K	AICc	Δ AICc	w_i	R ² m	R ² c
BQCV gene copies, individual <i>Bombus</i>	3	44.68	0	1.00	0.604	0.754
WB and HB BQCV abundance	3	58.78	14.10	0	0.400	0.551
Random effects	2	62.64	17.96	0	0	0.656
Null	1	79.06	34.38	0	0	NA

The summary of the top model showed that BQCV gene copies in the individual *Bombus* was significant and had a positive β value, suggesting a positive relationship between BQCV detection in pollen and BQCV quantity in each *Bombus* (Table 17). The model with site BQCV abundance also showed that BQCV abundance at the site was significant and had a positive relationship with BQCV detection in pollen. Each fixed effect from its respective model is plotted in Figure 13.

Table 17: Parameters of candidate models of parameters predicting BQCV presence in pollen. Coefficient (β value), standard error, z value, and corresponding p-value of each variable within each candidate model explaining BQCV presence in pollen.

Model	Parameter	β	Standard Error	z value	p-value
Individual BQCV	BQCV gene copies, individual <i>Bombus</i>	4.984	1.545	3.226	0.001**
	Intercept	-4.890	1.682	-2.906	0.003**
Site BQCV	WB and HB BQCV abundance	4.314	1.612	2.677	0.007**
	Intercept	-4.444	1.713	-2.594	0.009**

SBV in Pollen

The top performing model, as determined by using the function “dredge” on the global SBV model, was the model with only SBV abundance explaining SBV presence in pollen (Table 18). The next best model also included agricultural intensity as a fixed effect (Table 18), and these were the only models with a ΔAICc of less than 2 and that were included in the model average (Table 18). The variance of site was very small in the top and second highest models, 2.56×10^{-9} ($\pm$ SD 5.06×10^{-5}), and 2.87×10^{-9} ($\pm$ SD 5.35×10^{-5}) respectively, causing a singular fit warning for these models. Because of this, the marginal R^2 value was equal to the conditional R^2 value, so it was not included in the table. Site was retained as a random effect to account for the lack of independence between pollen samples collected from the same site.

Table 18: Initial model selection results of models explaining SBV presence in pollen. The function MuMIn::dredge was applied to the global model. The global model included presence/absence of SBV in each pollen sample as a response variable, fixed effects of SBV abundance of the wild and honey bees, percent agriculture, Julian date, and site as a random effect. Only models with a $\Delta\text{AICc} < 2$ were included in the table and in the model average. Differences in values from the highest ranked model (ΔAICc), the number of parameters in each model (K), Akaike weights (w_i), and marginal R squared values are included. Akaike weights (w_i) were taken from the model average, not the full table generated by “dredge”. A model containing only fixed effects and the null model are included for reference but were not included in the model average. All models except the null model include site as random effect.

Parameters	K	AICc	ΔAICc	w_i	R^2_m
WB and HB SBV abundance	3	34.38	0	0.58	0.591
WB and HB SBV abundance + % Agriculture	4	35.04	0.66	0.42	0.646

Only SBV abundance in bees was a significant predictor of SBV presence in pollen, with a positive β value indicating a positive relationship between SBV abundance and SBV detection in pollen (Table 19).

Table 19: Model average results of parameters predicting SBV presence in pollen. Coefficient (β value), standard error, z value, and corresponding p-value of each variable within the full model average explaining SBV presence in pollen. A model average was created with “model.avg” using models with a $\Delta AICc < 2$ after using “dredge” on the global model.

Parameter	β	Adjusted SE	z value	p-value
WB and HB SBV abundance	3.905	1.705	2.290	0.022*
% Agricultural	0.637	1.149	0.555	0.579
Intercept	-7.063	2.304	3.052	0.002**

Date and agricultural land use were not significant in the model average, so they were not included in candidate model set. In the candidate model set, the top ranked model was the model with SBV abundance as a fixed effect, performing over the model with SBV quantity in the individual pollen was from by a $\Delta AICc$ of 3.55 (Table 20). Both models performed better than the random effect and null models. The variance of site was again low at 1.50×10^{-8} ($\pm$ SD 1.23×10^{-4}) in the second-best model.

Table 20: Secondary model selection results of models explaining SBV presence in pollen. The function MuMIn::model.sel was applied to the candidate model set to compare the predictive power of SBV in the bee that pollen was collected from to SBV abundance in all collected bees on pollen SBV presence. Differences in values from the highest ranked model ($\Delta AICc$), the number of parameters in each model (K), Akaike weights (w_i), and marginal R squared vales are included. All models except the null model include site as random effect.

Parameters	K	AICc	$\Delta AICc$	w_i	R^2_m
WB and HB SBV abundance	3	34.38	0	0.90	0.591
SBV gene copies, individual <i>Bombus</i>	3	39.06	4.68	0.09	0.328
Random effects	2	43.79	9.41	0.01	0
Null	1	44.27	9.89	0.01	0

The β values of both SBV abundance in the bees from the site and SBV gene copies in the individual bee were positive and significant in their respective models, indicating they both

have a positive relationship with virus detection in pollen (Table 21). Each fixed effect from its respective model is plotted in Figure 13.

Table 21: Parameters of candidate models of parameters predicting SBV presence in pollen. Coefficient (β value), standard error, z value, and corresponding p-value of each variable within each candidate model explaining SBV presence in pollen.

Model	Parameter	β	Standard Error	z value	p-value
Site SBV	WB and HB SBV abundance	4.245	1.649	2.575	0.010*
	Intercept	-6.761	2.165	-3.122	0.002**
Individual SBV	SBV gene copies, individual <i>Bombus</i>	1.321	0.488	2.709	0.007**
	Intercept	-3.638	0.911	-3.993	<0.001***

DWV-B in Pollen

The top ranked model from applying the function “dredge” to the global model was the model containing only DWV-B abundance in the bees at the site as a fixed effect (Table 22). This was the only model with a $\Delta AICc$ of less than 2, so no model average was made, and only this model on its own was evaluated. Because this model is the same as one of the candidate models tested next, the model and coefficient summaries are displayed in Table 22 and Table 23. DWV-B abundance was a significant predictor of DWV-B presence in pollen, and because the β value is positive, there appears to be a positive relationship between DWV-B abundance and DWV-B presence in pollen. As seen in the SBV models above, the variance of site was very small in the top model, 1.17×10^{-9} ($\pm$ SD 3.43×10^{-5}), causing a singular fit warning. This also resulted in the marginal R^2 value to equal the conditional R^2 value, so it was not included in the table. Site was still retained as a random effect to account for the lack of independence between pollen samples collected from the same site.

Because agricultural intensity and date were not included in any model with a $\Delta\text{AICc} < 2$, they were not included in candidate models. The top ranked model was the model with only DWV-B abundance as a fixed effect, followed by the null model (Table 22). The model with DWV-B gene copies in the individual bee as a fixed effect performed worse than both the null and random effects models, and the variance of site was 4.51×10^{-9} ($\pm$ SD 6.72×10^{-5}). The random effect of site did not appear to improve the model, but it was retained to account for a lack of independence between pollen samples collected at the same site.

Table 22: Model selection results of models explaining DWV-B presence in pollen. The function MuMIn::model.sel was applied to the candidate model set to compare the predictive power of DWV-B in the bee that pollen was collected from to DWV-B abundance in all collected bees on pollen DWV-B presence. Differences in values from the highest ranked model (ΔAICc), the number of parameters in each model (K), Akaike weights (w_i), and marginal R squared values are included. All models except the null model include site as random effect.

Parameters	K	AICc	ΔAICc	w_i	R^2_m
WB and HB DWV-B abundance	3	20.41	0	0.88	0.464
Null	1	25.47	5.06	0.07	0
Random effects	2	27.62	7.21	0.02	0
DWV-B gene copies, individual <i>Bombus</i>	3	27.84	7.43	0.02	0.075

As previously discussed, DWV-B abundance was a positive, significant predictor of DWV-B presence in pollen in its model. Although the β value for DWV-B gene copies in the individual was also positive, it was not significant (Table 23). Each fixed effect from its respective model is plotted in Figure 13.

Table 23: Parameters of candidate models of parameters predicting DWV-B presence in pollen. Coefficient (β value), standard error, z value, and corresponding p-value of each variable within each candidate model explaining DWV-B presence in pollen.

Model	Parameter	β	Standard Error	z value	p-value
Site DWV-B	WB and HB DWV-B abundance	2.222	0.933	2.381	0.017*
	Intercept	-5.853	1.961	-2.985	0.003**
Individual DWV-B	DWV-B gene copies, individual <i>Bombus</i>	1.124	0.703	1.598	0.11
	Intercept	-3.171	0.695	-4.562	<0.001***

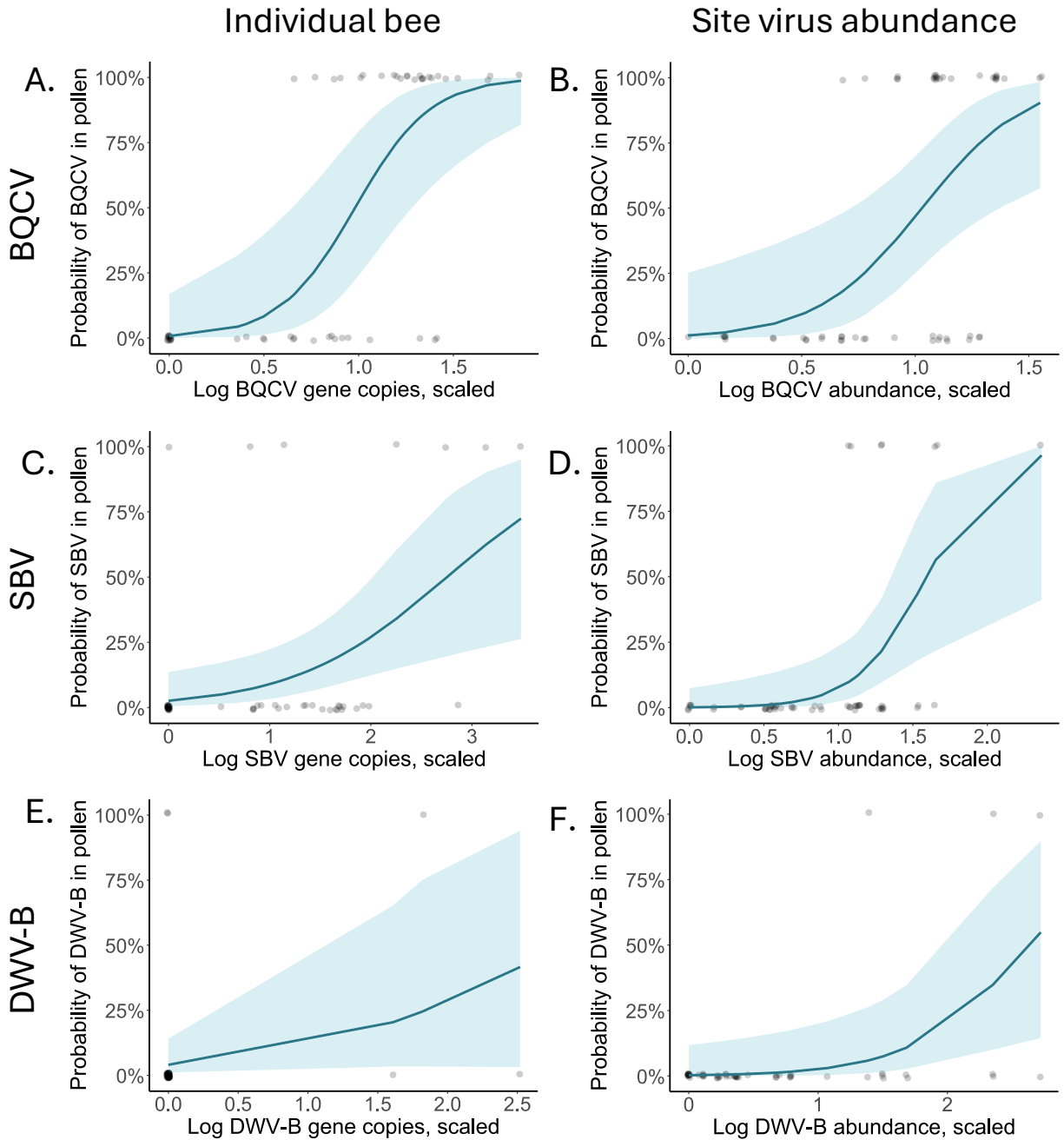


Figure 13: Adjusted predictions for the fixed effects of virus gene copies in the individual *Bombus* (A,C, E) and virus abundance at the site in honey bees and wild bees (B, D, F) in their respective models for BQCV (A, B), SBV (C, D) and DWV-B (E, F). Plots were created using the package “ggeffects” on the two candidate models for each virus with these fixed effects (Lüdtke 2018). The light blue areas represent 95% confidence interval. A jitter of 0.01 was included for readability.

DISCUSSION

Honey bee virus abundance and honey bee density

For all three viruses analyzed, BQCV, SBV, and DWV-B, the viral abundance measured in honey bees was a significant positive predictor of virus detections in wild bees from the same site. This lends evidence to our hypothesis that virus spillover from honey bees to wild bees is occurring. Most studies on virus spillover from honey bees have focused on bumble bees and report similar results, but our study extends this pattern to other bee families (Fürst *et al.* 2014; McMahon *et al.* 2015; Robson-Hyska 2017; Alger *et al.* 2019a). Recently, Maurer *et al.* (2024) found increased BQCV loads in honey bees were associated with increased BQCV loads in wild pollinators, although the same pattern was not observed for DWV-B. Piot *et al.* (2022) also found that for DWV, SBPV and the AKI-complex (which includes ABPV, Kashmir bee virus, and IAPV), virus prevalence in honey bees was correlated with virus prevalence in both bumble bees and solitary bees. Conversely, Melathopoulos *et al.* (2017) reported little evidence of virus spillover to managed *M. rotundata* in their study, although SBV presence in adult *M. rotundata* at the site was associated with reduced cocoon viability.

Although direction cannot be inferred from our results alone, viral prevalence and intensity (when comparable) were higher in honey bees than in wild bees, suggesting that honey bees are generally the primary hosts of these viruses within our study system. This pattern is consistent across many studies investigating these viruses, although some studies have found no difference in prevalence and/or intensity between honey bees and wild bees for certain viruses (Fürst *et al.* 2014; McMahon *et al.* 2015; Dolezal *et al.* 2016; Robson-Hyska 2017; Radzevičiūtė *et al.* 2017; Alger *et al.* 2019a; Jones *et al.* 2021; Wham *et al.* 2024; Pfeiffer *et al.* 2024).

Important to note is that we only measured the presence and number of gene copies of a virus in

each bee. These measurements do not confirm virus infections, which are better verified via the detection of the negative or replicative viral strand (Radzevičiūtė *et al.* 2017; Warncke and Knudsen 2022).

Honey bee density during the time of sampling was not significant in predicting the presence of any virus in wild bees. Studies have reported that apiary presence, distance, or honey bee density are significant in predicting patterns of virus prevalence for some viruses, but others have found this not to be the case, or only the case for certain viruses (Fürst *et al.* 2014; McMahon *et al.* 2015; Alger *et al.* 2019a; Tiritelli *et al.* 2024; Maurer *et al.* 2024; Kahnnonitch *et al.* 2025). This indicates that honey bee density alone is not necessarily driving virus spillover, and viral load in foraging honey bees may be the more important factor, at least in our system. Proesmans *et al.* (2026) reported similar results, where the main driver of BQCV and DWV prevalences in wild pollinators was honey bee viral loads or prevalence, and not honey bee density.

With this in mind, limiting pathogen spillover to wild bees could be most effectively achieved by treating honey bees for pathogens. As *Varroa* mites play a large role in the spread of DWV and IAPV between honey bees, employing effective management strategies of mites could benefit honey bee and wild bee health (Gisder *et al.* 2009; Di Prisco *et al.* 2011; Locke *et al.* 2012; McMenamin and Genersch 2015; Manley *et al.* 2019). This is highlighted by the study performed by Manley *et al.* (2019) on a series of islands, some of which are free of *Varroa*, which found that DWV prevalence and intensity in both honey bees and bumble bees are primarily driven by *Varroa* mite presence. Issues of acaricide resistance in *Varroa* mites have been pervasive in the last few decades, so the continual development of new mite treatments and employing strategies to limit promoting resistant *Varroa* strains is essential (Mitton *et al.* 2022;

Lester 2023). Although mite treatments are often simpler to manufacture and apply, directly treating colonies for viruses is also an emerging possibility. Treatments for viruses fed to colonies are being developed using double-stranded RNA or antibodies, and this strategy could be more effective for viruses with less clear associations with *V. destructor* including SBV and BQCV (Hunter *et al.* 2010; Desai *et al.* 2012; Piot *et al.* 2015; Yang *et al.* 2018; MacMillan *et al.* 2024). Because these treatments are likely one of the easier ways to prevent pathogen spillover to wild bees, continued research into treatments for *Varroa* and viruses should be a priority.

Agriculture and floral metrics

In Canada, 97% of the prairies' ecozone was classified as agriculture as of 1996 (Environment Canada 1996), and land used for crops increased by 38% between 1961-1966 and 1991-1996 (Carlyle 2002). In the prairies, the area used for canola cultivation alone increased by 700% between 1976 and 1996 (Environment Canada 1996), and our study was conducted in this intensively managed landscape. Wild bee diversity, species richness, and density (mean wild bees per transect) all tended to be higher at "non-agricultural" sites when compared to those located on canola fields (Table 4). This is to be expected when sampling in an area with a lower diversity of flowering species, where most of the area is dominated by a single flowering crop. While 63 wild bee species were tested for viruses, across all transects 95 different non-*Apis* species were recorded, capturing almost a quarter of the bee diversity found in Manitoba (Gibbs *et al.* 2023). Our study found an expected diversity based on sampling effort, as only a total of 505 wild bees and 378 honey bees were collected in transects. For reference, a study performed by Killewald *et al.* (2025) sampling in and around agricultural fields and at semi-natural sites in Manitoba found 140 bee species when employing three different sampling methods, capturing a total of 12,786 bees.

The proportion of agriculture surrounding each site was observed to increase the risk of both BQCV and SBV transfer to wild bees, and it was additionally the only variable found to affect wild bee diversity. The negative effect of agriculture on wild bee diversity has previously been documented by Koh *et al.* (2016) on a larger scale spanning the United States, so our result is unsurprising. This reduced diversity and increased virus transfer risk could be due to a combination of factors, including low floral diversity and pesticide use. Additionally, a study involving honey bees found that the number of stressors colonies faced, included pesticides, parasites, bacteria, and viruses, increased after exposure to crops, stressors that wild bees also face (French *et al.* 2024). Although our study did not detect any effects of virus abundance in honey bees on wild bee diversity, it is possible that in our analysis the association of virus abundance and wild bee diversity exists but was not detectable. This could be due to our smaller sample size of only 20 sites and due to the use of only one trapping method (netting), whereas other studies report that combining trapping techniques is best for accurately estimating bee diversity (Prendergast *et al.* 2020; Pei *et al.* 2022). Effects of viruses on wild bees, if present, also may only be detected with sustained, multi-year contact between honey bees and wild bees. Some sites used in this study had low honey bee densities despite having honey bee hives present in previous years. A study by Cohen *et al.* (2022) found that in areas with higher bee diversity, pathogen richness was decreased, which they attributed to a dilution effect with increased diversity. Fearon and Tibbetts (2021) detected a similar relationship in their study, where greater bee species richness was associated with lower prevalences of DWV, SBV, and BQCV, which they also attributed to pathogen dilution. It is also possible that in these relationships, greater pathogen richness or prevalence is driving lower bee diversity, but determining the direction of this relationship would require experimental manipulation and further research.

Our agricultural sites were situated on canola fields, and when in bloom this large monocrop meant that floral resources available to bees were quite homogenous. This could result in a greater floral resource overlap between honey bee and wild bees both foraging on canola, increasing virus transfer risk. The virus transfer risk posed by resource overlap was demonstrated by Maurer *et al.* (2024), who showed that floral resource overlap with honey bees was a significant predictor of both BQCV and DWV-B prevalence and load in wild pollinators. Cohen *et al.* (2021) similarly found that bee abundance increased with large monocrop blooms, where limited flowering species choices would be expected to increase resource overlaps between bees, and bee abundance was positively associated with parasite prevalence.

In our study, the floral resources available to bees could also affect the nutritional value of the pollen they ingest. Although canola is considered to be a nutritious source of pollen for bees, the diversity of pollen collected at more agriculturally intensive sites is likely low (Pernal and Currie 2000; Cook *et al.* 2003). High pollen diversity has been shown to boost certain immune functions and has been associated with lower virus intensities in honey bees (Alaux *et al.* 2010; Antúnez *et al.* 2015). This could be contributing to the effect of agriculture observed here, where high pollen diversity reduces virus susceptibility in bees caught in less agriculturally intense areas. Although no studies examine how pollen diversity affects immunity in non-*Apis* bees, a study by Manley *et al.* (2017) did find that nutritional stress from starvation increases the virulence of SBPV in *B. terrestris*. A study by Hsieh and Dolezal (2024) found that certain pesticides further increased honey bee mortality when they were infected with IAPV compared to those not treated with pesticides, but only when they were fed a protein supplement. Conversely, when the bees were fed pollen, no difference in mortality was seen between IAPV infected bees treated or untreated with pesticides, and in one case the pesticide-treated bees even

had an increased survivorship. Complex interactions between pesticides and nutrition can contribute to virus susceptibility and transmission in bees, so both factors should be considered potential risks for agriculture.

Alone, many pesticide classes have been shown to have a variety of negative impacts on bee health, effects that have been best studied in honey bees, but these effects have also been observed in other bee species (Thompson 2003). Effects such as increased mortality, behavioural changes, and impacted learning and memory have been measured in honey bees (Thompson 2003; Wu *et al.* 2011; Henry *et al.* 2012; Frost *et al.* 2013; Siviter *et al.* 2018), and pesticide use has been associated with wild bee declines (Park *et al.* 2015; Janousek *et al.* 2023; Guzman *et al.* 2024) and mass fatalities (Hatfield *et al.* 2021). Viral titres in honey bees have also been found to increase with pesticide exposure, and synergistic effects of viruses and pesticides can increase honey bee mortality (Di Prisco *et al.* 2013; Doublet *et al.* 2015; Degrandi-Hoffman *et al.* 2015; Sánchez-Bayo *et al.* 2016; Fine *et al.* 2017; Coulon *et al.* 2018; Hsieh and Dolezal 2024). As pesticides increase virus susceptibility in honey bees through immune suppression, it is likely that this also occurs in wild bees (Di Prisco *et al.* 2013; Sánchez-Bayo *et al.* 2016; Fine *et al.* 2017). In agriculturally intensive areas, pesticides could increase infection intensity in honey bees, increase virus transmission among honey bees, and increase the transmission risk to wild bees.

Measures of floral species richness and floral abundance were not significant apart from the association of floral abundance and DWV-B detections in wild bees. Although no issues of collinearity were detected in our models, some variation in floral metrics may have been captured by agricultural land use, and vice versa. Increased floral abundance was expected to reduce transfer due to a dilution effect, reducing resource overlap between bees with more

flowers available, but the opposite effect was seen in DWV-B. Floral abundance, measured as the average percent of quadrat areas covered with flowers, was highly associated with canola bloom in particular (3.8% – 31.9%), with floral abundance remaining comparatively low outside this time and at semi-natural sites (<0.01% – 3.5%). This potentially could be why floral abundance was a significant positive predictor of DWV-B detections, as DWV-B transfer could be higher during canola bloom at sites situated on canola fields. This may be due to the variety of the factors associated with agriculture discussed above. For example, as canola is the main resource present during its bloom, low pollen diversity could contribute to reduced immune function during this period. Also, many bees may be attracted to canola fields during their mass flowering, since there are many flowers of one species present there could be an increase in resource overlap between honey bees and wild bees. As discussed above, Cohen *et al.* (2021) found that when few non-crop flowers were present at sites, large blooms of monocrops increased bee abundance, which was associated with greater parasite prevalence. On the other hand, non-crop floral abundance was associated with reduced parasite prevalence (Cohen *et al.* 2021). This parasite reduction with increased non-crop flowering plants could potentially be due to decreased resource overlap between bees, where bee species select different flower species to forage from, spreading out across diverse resources and reducing transfer risk (Cohen *et al.* 2021).

To mitigate the effects of agriculture on pathogen spillover and wild bee diversity, one potential strategy could be to plant flower strips adjacent to agricultural fields, increasing the number of non-crop flowers present. While having the potential to reduce resource overlap between bee species, flower strips can additionally provide a diverse range of pollen for both honey bees and wild bees, which could improve bee immune function and reduce virus

susceptibility (Alaux *et al.* 2010; Antúnez *et al.* 2015). Flower strips have also been shown to increase bee richness and abundance in field margins, which could help counter the negative impact we observed of agriculture on wild bee diversity (Buhk *et al.* 2018; Zamorano *et al.* 2020). This strategy can also be advantageous to farmers with crops that benefit from pollination, as flower strips have been shown to increase bee visitations and abundance in adjacent fields (Feltham *et al.* 2015; Ganser *et al.* 2018; Killewald *et al.* 2025). Flower strips have also been found to increase the spillover of natural enemies of pests into fields (Tschumi *et al.* 2016; Woodcock *et al.* 2016). This could help suppress crop pests and decrease the need for pesticide applications, which could also be beneficial for bees (Kowalska *et al.* 2022). To ensure these plantings have a positive impact on pollinators, Cohen *et al.* (2022) suggests to specifically focus on flowering species that promote bee diversity, which is associated with lower pathogen richness and prevalence (Fearon and Tibbetts 2021). They also suggest choosing flowers and that limit pathogen spillover, as various floral traits are likely to impact pathogen transmission (McArt *et al.* 2014; Adler *et al.* 2018; Cohen *et al.* 2022; Pinilla-Gallego *et al.* 2022; Maurer *et al.* 2024). A beneficial flower strip in this context is one that provides a diversity of flowers to reduce resource overlap between bees, increases pollen diversity, and promotes bee diversity and pathogen dilution.

Phylogenetic relatedness of wild bees to honey bees

Our findings showed that wild bee species more closely related to honey bees are more likely to be detected with BQCV and SBV than those more distantly related. Although this has been discussed as a possibility for honey bee viruses (Manley *et al.* 2015; Sagastume *et al.* 2025), this is the first study demonstrating this pattern when studying virus detections across many bee species. In mammalian hosts, more closely related species are more likely to share

viruses, so the pattern we observed in bees matches our expectations (Wells *et al.* 2020). In bees, Dalmon *et al.* (2021) found that the effect of the family Apidae on SBV prevalence was strongly positive compared to other bees families. Doublet *et al.* (2025) also demonstrated that bee phylogenies closely follow patterns of virome dissimilarity, so host genetics had the strongest influence on the communities of insect viruses found in each species. Fearon and Tibbetts (2021) noted a similar pattern to the relationship we found, where viral prevalence in three wild bee species they tested appeared to follow a pattern of relatedness to honey bees. Interestingly, they also noted that while BQCV and SBV tend to follow phylogenetic patterns of infection in relation to honey bees, DWV appears to be a more generalist virus, infecting a broader range of hosts (Fearon and Tibbetts 2021). This is consistent with our results, which showed no significant effect of phylogenetic distance from honey bees on DWV detections in wild bees, although DWV-B and DWV-A prevalence and DWV-B intensity was greater in honey bees compared to wild bees. DWV could be an important virus to further study in other bee hosts, regardless of their relation to honey bees.

Based on these findings, future studies on BQCV and SBV should focus on more thoroughly testing bee species closely related to honey bees for virus susceptibility and health impacts, including how they interact with other stressors in these groups. Aside from *Apis* species, bumble bees are the next most studied genus of bees in terms of bee viruses. This could be due to their effectiveness as commercial pollinators, the ease of acquiring and rearing colonies, their charismatic status, or their close relationship with honey bees, which results in greater virus susceptibility. *Bombus* was the most closely related genus of bees to honey bees collected in this study, and along with the tribe Meliponini which is present closer to the equator, are the closest extant relatives to the genus *Apis* (Hedtke *et al.* 2013; Henríquez-Piskulich *et al.*

2024). Most infection-based studies outside of *Apis* have been conducted on *Bombus*, and this work should expand, to test more viruses and potential sublethal viral effects.

Meliponini, a tribe commonly known as stingless bees, are an important group both culturally and economically for their honey production, and ecologically for pollination (Grüter 2020a, 2020b). These diverse bees are very common in tropical climates, accounting for 50% of bee visits to flowers across five studies in tropical America (Grüter 2020b). Although many studies have detected honey bee viruses in various stingless bee species, few have focused on direct impacts (Morfin *et al.* 2021b; Toledo-Hernández *et al.* 2022; Fleites-Ayil *et al.* 2023). One study experimentally inoculated *M. beecheii* individuals with sugar syrup containing field-realistic doses of DWV-A, DWV-B, or BQCV (based on their findings in the species), and found that the survival of bees infected with any of the three viruses was significantly reduced compared to those fed sugar syrup with buffer (Fleites-Ayil *et al.* 2023). More research is needed to understand the impact these viruses have across stingless bee species, and how negative impacts can be mitigated.

Along with *Bombus* and Meliponini, other bee species within the family Apidae, and species in Megachilidae, the sister family to Apidae, should be the focus of future virus spillover research due to their close relationship to honey bees. Only one study has detected effects of honey bee viruses on bees outside of Apidae (Singh 2011), but few studies have tested for them (Dolezal *et al.* 2016; Schauer *et al.* 2023). *M. rotundata*, the commercially managed alfalfa leafcutter bee, was found to have a shortened lifespan when fed IAPV as an adult, and was more likely to emerge as an adult instead of entering diapause when fed IAPV as a larva (Singh 2011). Emphasizing the need for this research, the two wild bees from our study with the highest

number of BQCV gene copies were from Megachilidae: a male *Heriades carinata* (1.3×10^8 gene copies/ μg RNA), followed by a female *Hoplitis producta* (4.8×10^7 gene copies/ μg RNA).

Sociality and sex

Kleptoparasitic bees were more likely to be detected with SBV when compared to eusocial and solitary bees. This could be because kleptoparasitic bees, also known as cuckoo bees, have a facultative need to interact with other bee species at multiple life stages (Bogusch *et al.* 2006). The cuckoo bees we tested in this study were from the genera *Stelis*, *Nomada*, and *Epeolus*. Adults of these species lay their eggs in cells within the nests of other bee species, and when these eggs hatch, the kleptoparasitic larvae kill the young occupying these cells and consume their provisioned food (Rozen and Kamel 2009; Litman *et al.* 2013). This could potentially provide opportunities for these bees to obtain viruses if SBV can be transmitted between wild bee species. A study by Tiritelli *et al.* (2024) observed a similar effect of kleptoparasitism on virus risk; cuckoo bees were at a greater risk of DWV and ABPV infections. A study by Ranalli *et al.* (2026) focused on cuckoo wasps (Hymenoptera: Chrysididae), many of which are kleptoparasites of bee and wasp species, also found that there was a high overlap of honey bee-associated pathogens, including viruses, found in cuckoo wasps and their respective bee and wasp hosts.

While we found that cuckoo bees were at an increased risk of SBV, we did not detect differences in virus transfer risk between eusocial and solitary bees. Although living in a colony with other bees might be expected to increase pathogen transmission risk, social bees may benefit from group behaviours that reduce their susceptibility, referred to as social immunity, which could counteract the risks associated with eusociality (Meunier 2015; Durand *et al.* 2023; Tiritelli *et al.* 2024). Other studies have also reported no differences in virus risk when

comparing eusocial to solitary bee species, so our results match these findings (Dalmon *et al.* 2021; Tiritelli *et al.* 2024).

Sex was also not a significant predictor of virus presence in wild bees. We hypothesized that female bees may be at a greater risk because they collect pollen to provision their offspring (Thorp 2000; Ne'eman *et al.* 2006), but this could potentially be offset by behaviours in males. Male bees of many species tend to spend the night outside nests, and certain species sleep on flowers during the night (Welsford and Johnson 2012; Pinheiro *et al.* 2017), potentially exposing males to viruses deposited on flowers. Males of many species also tend to be active later in the season (Torchio and Tepedino 1980; Ogilvie and Thomson 2015), when we found virus transfer risk was elevated for BQCV and SBV. These behaviours could make the risks faced by males similar to those faced by foraging females. Our results are in line with those found by Figueroa *et al.* (2021), which found no differences in pathogen prevalence between bee sexes.

Seasonal viral dynamics

The chance of detecting BQCV and SBV in wild bees increased over the course of the summer. Seasonal trends in viruses have been reported in other studies, and although most focus on honey bees, a study by Wham *et al.* (2024) found that seasonal virus trends were similar in honey bees and bumble bees. Counter to our findings, Robson-Hyska's (2017) study in Manitoba found that in bumble bees, virus prevalences of DWV, BQCV, SBV, and IAPV appeared to decrease over the course of the summer in bumble bees, especially in populations sampled from areas without honey bees present, except in the case of BQCV in the presence of honey bees. Often in honey bee colonies, studies report either an increase in BQCV prevalence from spring to summer (Tentcheva *et al.* 2004; de Miranda *et al.* 2013; Wham *et al.* 2024), which matches what our study found in wild bees, but others report little seasonal variation in prevalence as it is

always high (Robson-Hyska 2017; Avci *et al.* 2022; Molinatto *et al.* 2025). All honey bees we tested had detectable BQCV, so this is unsurprising, but prevalence in wild bees appears to follow a more distinct trend of increasing over the season. SBV in honey bees has been reported to either increase from spring to summer (Tentcheva *et al.* 2004; Molinatto *et al.* 2025), which matches the patterns we observed in wild bees, or as peaking in the spring then falling which we did not observe (de Miranda *et al.* 2013). Many studies also report DWV prevalence as increasing from spring to fall, but we did not observe any effect of date on wild bee DWV-B detections in wild bees (Tentcheva *et al.* 2004; de Miranda *et al.* 2013; Traynor *et al.* 2016; Wham *et al.* 2024; Molinatto *et al.* 2025). Because there were only 35 detections of DWV-B in wild bees, this study may have limited power in detecting weaker trends that would be seen if this study were performed in an area with a higher prevalence of DWV-B in both honey bees and wild bees. As DWV-B tends to peak in fall when *Varroa* mites are more prevalent, we also may not have sampled late enough in the season to detect this trend (Traynor *et al.* 2016; Molinatto *et al.* 2025).

Seasonal trends in virus detections could also be related to a number of other factors. French *et al.* (2024) found that the number of stressors detected in honey bee colonies increases across the field season at sites with commodity canola, which makes up of most of our canola sites. These stressors included pesticides, parasites, bacteria, and viruses, many of which could also impact the wild bees at field sites and could directly or indirectly influence viral dynamics (French *et al.* 2024). Increased prevalence of BQCV and SBV over the summer also follows the pattern of seasonal honey bee colony growth, as populations begin low in the spring and peak in late summer, meaning there could be more honey bees present transmitting viruses (Farrar 1937). It is possible that the increase in honey bee populations across the field season within hives

influences seasonal BQCV and SBV dynamics in wild bees, although we did not see any direct effect of honey bee density on wild bee virus presence. Alternatively, BQCV and SBV may build up in wild bee populations during the summer while they are in contact with honey bees, and conspecifics in the case of eusocial species, then drop during the winter once this contact ends, creating an annual cyclical pattern of transmission risk.

Viral prevalence and co-occurrences

The most prevalent virus found in our study was BQCV, which was found in all honey bee samples and in almost half of wild bees. Other studies have frequently reported high prevalences of BQCV, with BQCV as the most or second most prevalent virus detected, although studies testing individual bees (without pooling samples) generally don't find that all honey bees harbour BQCV (Singh *et al.* 2010; D'Alvise *et al.* 2019; Fleites-Ayil *et al.* 2023; Maurer *et al.* 2024). SBV was found in over two-thirds of honey bees in our study and in almost a quarter of wild bees. Often SBV is reported as having a lower prevalence than we found (Singh *et al.* 2010; McMahon *et al.* 2015; D'Alvise *et al.* 2019), although Dolezal *et al.* (2016) report prevalences of 71% in field-collected honey bees and 45% in wild bees, which is slightly higher than our study. DWV had a lower prevalence compared to some studies (Welch *et al.* 2009; Singh *et al.* 2010; Maurer *et al.* 2024), but other studies report prevalences similar to ours (McMahon *et al.* 2015; D'Alvise *et al.* 2019; Wham *et al.* 2024). DWV is often one of the most prevalent viruses found in honey bees, and is sometimes even more prevalent than BQCV, which we did not observe (McMahon *et al.* 2015; Jones *et al.* 2021; Tiritelli *et al.* 2024). Consistent with our findings, DWV-A alone often does have a low prevalence in more recent studies that separate the two variants, as DWV-B and DWV-A/B recombinants have become the dominant variants since the emergence of DWV-B (Paxton *et al.* 2022; Maurer *et al.* 2024; Dobelmann and Wilfert 2025;

Sircoulomb *et al.* 2025). The somewhat lower prevalence of DWV in our study could be due to a variety of factors, including geographic location and annual viral dynamics, or due to effective Varroa mite management by beekeepers, as multiple sites with honey bees present were managed by the same beekeepers. IAPV also had relatively low prevalence, consistent with most other studies (Singh *et al.* 2010; Wham *et al.* 2024), although counter to what Robson-Hyska (2017) found in Manitoba.

Viral co-occurrences were commonly detected in our study, with most honey bees (82.1%) and 25.0% of wild bees having two or more detectable viruses. Co-occurrences are to be expected, as many other studies have reported virus coinfections in both honey bees and wild bees (Berényi *et al.* 2006; McMahon *et al.* 2015; Manley *et al.* 2020; Nanetti *et al.* 2021; Jones *et al.* 2021; Cilia *et al.* 2022; Durand *et al.* 2023; Tiritelli *et al.* 2024). The rate of coinfections in honey bees found by Berényi *et al.* (2006) in a survey of six viruses closely reflects the rates we detected in honey bees. Numerous co-occurrences of viruses in wild bees are also unsurprising, as Tiritelli *et al.* (2024) found that 40.5% of all wild bees they sampled were co-infected with 2 or more pathogens in their study evaluating ten common honey bee pathogens, including five viruses.

Phylogeny and dead-end spillover

Sacbrood virus sequences from this study showed no evidence of clustering by host species, and instead sequences from honey bees were interspersed with those from wild bees. This pattern was also observed in the other four viruses, and for all viruses except DWV-A, at least one identical sequence was found in both a wild bee and honey bee. Most SBV sequences obtained from GenBank that could be aligned with our samples were from *A. mellifera* or *A. cerana*, and together they showed a general trend of clustering based on geographic location.

This pattern was observable despite the extensive international trade of honey bees that occurs every year, and even though viruses are often not considered when restrictions are placed upon honey bee colony imports and exports (Matheson 2000, Mutinelli 2011, World Organisation for Animal Health 2024). This pattern is unsurprising, as the same geographic pattern has been reported by other studies on honey bee viruses (Radzevičiūtė *et al.* 2017; Murray *et al.* 2019; Manley *et al.* 2020; Chantaphanwattana *et al.* 2023).

As observed in our samples, many studies have found that honey bee viruses do not form distinct clusters in wild bee populations and share high sequence similarities with viruses found in honey bees (Singh *et al.* 2010; Levitt *et al.* 2013; Murray *et al.* 2019; Manley *et al.* 2020; Dalmon *et al.* 2021; McKeown *et al.* 2025). In a multi-year survey of honey bee and bumble bee viromes, McKeown *et al.* (2025) expressed that their results indicate that virus spillover to bumble bees is a case of dead-end spillover, as these viruses are not diversifying within wild bees to indicate true host expansion. This appears to be the case in most other studies, including ours, where spillover is likely due to environmental exposure rather than host expansion. One exception was found when studying virus spillover to stingless bees in Mexico, where a haplotype of BQCV was found exclusively in *M. beecheii* while other haplotypes were shared between *M. beecheii* and *A. mellifera* (Fleites-Ayil *et al.* 2023).

According to McKeown *et al.* (2025), in cases of host expansion in wild bees, transmission between wild bee species would be expected in conjunction with diversification within a species. When studying virus transmission between bees with shared food sources, Tehel *et al.* (2022) found that bumble bees are unlikely to transmit DWV-A to other bumble bees or to honey bees when infected, despite the clear evidence that honey bees readily transmit the same strain. Another study by Streicher *et al.* (2024) found that bumble bees injected with

DWV-B shed less virus in their feces compared to honey bees, and bumble bees fed the virus do not have detectable DWV-B in their hemolymph, while honey bees and injected bumble bees do. These results indicate that bumble bees may not be transmitting DWV to other bees if they are infected through feeding. Still, the possibility that viruses can differ in their ecology should be considered, and other viruses may be more easily transmitted by wild bee species and should be investigated.

Wild bees that are dead-end hosts could still be negatively impacted if they are susceptible to viral infections. In cases where humans are dead-end hosts, hundreds of thousands of human deaths have occurred due to RNA virus spillover from bats and mice to humans (Williams *et al.* 2021). With sustained contact between honey bees and wild bees, a health risk due to environmental exposure could be posed, particularly for susceptible wild bee groups. Additionally, with increased contact between honey bees and wild bees, there is an increasing risk of a spillover event that establishes a virus in a wild bee population leading to true host expansion. This could increase the virulence of a virus, cause it to spread between wild bees, and potentially spill back into honey bees. More interactions with a potential host gives more opportunities for pathogen spillover and provides a higher chance of a successful spillover event, a pattern that has been seen when examining spillover events from wildlife to humans (Saldaña *et al.* 2024). In general, reducing spillover risk between species begins with addressing ecological issues including habitat loss, agricultural intensification, and climate change (Saldaña *et al.* 2024).

Sequence divergence

There was a difference in sequence variation observed between BQCV and all other viruses which was interesting to note. Although sequence lengths were variable and sample sizes

were too small for many viruses to draw conclusions, it was noted that BQCV had an average sequence divergence between five and nine times smaller when compared to the other viruses. This could be due to approximately half of the targeted area being outside of an open reading frame (when aligned with the reference genome, NC_003784.1), or due to another mechanism keeping this area highly conserved. It should be noted that all other virus primers targeted areas fully within an open reading frame of each virus (when aligned to reference genomes, see SBV: NC_004830.2, DWV-B: NC_006494.1, DWV-A: NC_004830.2, IAPV: NC_009025.1).

Protocol recommendations and limitations

When creating a phylogeny of SBV sequences, it was noted that the SBV sequence used in this study as a standard curve (gBlock™) matched with a sequence from Vietnam (KC007374.1), which is relatively divergent from the sequences detected in this study. For future studies, we recommend using a sequence from the continent of interest, as a gBlock™ standard could yield more accurate quantification results when sequence similarity between the unknowns and the standard is higher.

Other studies have reported issues using of finding reference genes for an internal control that function across a range of bee and arthropod species (Levitt *et al.* 2013; McMahon *et al.* 2015) and for our purposes, when we tested actin and primers used in other studies (Radzevičiūtė *et al.* 2017), we had limited success. Most tested primers pairs used either by other studies (Opsin, CO1, β -Actin), or chosen as potential candidates for testing (GAPDH, GAPDH HB, S18) included at least one bee sample that did not meet the detection threshold, and often there were great differences in peak melt temperatures between bee species. The primer set for the ribosomal gene S28, designed by Sankar *et al.* (2024) based on *Bombus terrestris*, was the only set to successfully detect every bee sample and display consistent peak melt temperatures

between the bee species tested, although there was species based variation in Cq values. Although it was found that there was some variation in peak melt temperatures once this primer set was used as an internal control for all study samples, Cq values and peak melt temperatures were consistent within species. This primer set is a good candidate to use in future studies requiring an internal control across many species, with the exception of the genus *Colletes* for which using primers for Opsin (Almeida and Danforth 2009; Radzevičiūtė *et al.* 2017) could be substituted based on our low success using S28. Additionally, two other studies have used different pairs of S28 primers as internal controls across a variety of bee (Jones *et al.* 2021) and bee and hoverfly species (Maurer *et al.* 2024), although fewer species (3 and 20) were included in these studies compared to ours.

It should be noted that honey bee density estimates may have been less accurate in our study due to our sampling regime. Transects were performed where there was the highest density of flowers, which during canola bloom was within canola fields at “agricultural” sites. Sometimes, especially when honey bee density appeared lower, it was noted that there were visibly more honey bees and wild bees foraging on flowers like clovers in the field margins, despite the higher floral density in the canola fields. This may have contributed to inaccurate honey bee estimates that otherwise would have produced different results. Despite this, when honey bee density appeared highest, generally when sampling at sites 250m from known apiaries, there were many honey bees captured foraging on canola. Additionally, honey bee density measures are influenced by weather conditions, and although sampling was performed when conditions met our criteria, some days and times of day were likely better for sampling than others due to less cloud cover, warmer temperatures, and calmer winds.

Pollen and pathogen spillover

We found that viruses were frequently detected in pollen samples, and site-level virus abundance was a significant predictor of virus presence in pollen samples. We also found two pollen samples with detectable DWV-B when DWV-B was not found in the respective bumble bee, and a SBV pollen sample where this was the case. These results support our hypothesis that wild bees are collecting pollen with viruses present, and these viruses were deposited on flowers by other bees present at the site. This matches results reported by Singh *et al.* (2010), where uninfected honey bee foragers had detectable DWV and SBV in their pollen loads. Although both our studies did not find uninfected bees with pollen containing BQCV, it is difficult to tell if this is due to the high prevalence of BQCV in the tested bees (98.5% of honey bees by Singh *et al.* (2010), 75% of bumble bees in this study), or because of a difference in viral ecology between BQCV and other viruses. Additionally, both our studies found that bees with detectable virus often carry pollen loads without that same virus present, which could mean that the process of collecting pollen doesn't necessarily contaminate the pollen with virus. In this case, saliva and regurgitation during pollen collection are likely not the main mechanisms causing pollen contamination, and another pathway is responsible. Singh *et al.* (2010) also found that viral sequences between a forager and her pollen often did not match, even when they were detected with the same virus present, which is another piece of evidence against saliva as the main transfer pathway. Singh *et al.* (2010) hypothesizes that this pathway is likely through defecation because multiple viruses have been detected in honey bee feces (Bailey and Gibbs 1964; Hung 2000; Chen *et al.* 2006b, 2014; Holmes *et al.* 2026), bumble bees defecate while foraging (Bodden *et al.* 2019), virus quantities have been found to be higher in honey bee digestive tracts

compared to other tissues (Chen *et al.* 2006b), and honey bee feces with CBPV particles is infective (Ribi  re *et al.* 2007).

Our study was also able to compare if site-level virus abundance (combining all honey bees and wild bees) or the virus in individual bumble bees best predicts virus presence in pollen. For SBV and DWV-B, the expected pattern was found, where the site-level virus abundance was the better predictor. This would be expected if the bees present at a site are contributing to the viruses present in pollen through traffic on flowers, instead of viruses coming only from the bee that collected that pollen load. Conversely, although virus abundance at the site was a significant predictor, BQCV presence in pollen was better predicted by the viral gene copies in the individual that collected the pollen. This could mean that the viral ecology of BQCV is different than SBV and DWV-B, and that transmission between these two groups cannot be equated. Saliva or other secretions associated with nectar regurgitation may contribute to BQCV transfer, although it is important to note that many pollen samples without BQCV came from bumble bees with detectable BQCV. SBV and DWV-B belong to the family Iflaviridae, while BQCV belongs to the family Dicistroviridae, so it is possible that these differences in ecology could be partially explained by shared adaptations. If we had more data, it would be interesting to compare IAPV to these viruses, as it is also within the family Dicistroviridae. Laboratory experiments that have demonstrated virus transmission between honey bees and bumble bees commonly use just DWV as the virus of interest (Burnham *et al.* 2021; Tehel *et al.* 2022), and studies less often include other viruses (Singh *et al.* 2010; Alger *et al.* 2019b). In the lab, Tehel *et al.* (2022) found that bees feeding on the same food source can result in DWV-A transmission from honey bees to bumble bees, but found no evidence that transmission was bi-directional. Similarly, Streicher *et al.* (2024) found infected honey bees to shed more DWV-B in their feces compared to *Bombus*

terrestris. On the other hand, Singh *et al.* (2010) found in their greenhouse experiment that IAPV could be transmitted both from honey bee to bumble bee colonies foraging on the same flowers, and from bumble bee to honey bee colonies. It is possible that certain viruses, including DWV and possibly SBV, are not commonly transmitted by bumble bees, while viruses like BQCV and IAPV can be. Alger *et al.* (2019b) did find differences in how DWV and BQCV were deposited by foraging honey bees across flower species, hinting at potential ecological differences between these two viruses. Although this paper did not find evidence of subsequent bumble bee infections, a more recent paper from the same group of researchers (Burnham *et al.* 2021) did find evidence of DWV transmission from honey bees to bumble bees sharing flowers. A controlled laboratory experiment, comparing transmission differences between viruses when bees share food sources, could help clarify these potential differences in viral ecology.

As demonstrated, pollen collected directly from flowers (Mazzei *et al.* 2014) and honey bee hives (Singh *et al.* 2010) can be infective, so care should be taken when collecting pollen used to feed bee colonies. Commercial bumble bee colonies used for greenhouse pollination are often reared on pollen collected by honey bee foragers (Meeus *et al.* 2014b). Honey bee collected pollen is also sometimes used to supplement honey bee feed when there is a lack of available forage (Johansson and Johansson 1977; Mattila and Otis 2006). If this pollen is infective, it could introduce viral infections into colonies and negatively affect their health. To avoid this issue, techniques like gamma irradiation, which is commonly used to kill microbes in food consumed by humans (Sommers 2012), can be used on pollen prior to using it as feed (Graystock *et al.* 2016a). This has been shown to successfully reduce or inactivate viruses and other bee pathogens, and gamma irradiation is already used by some companies to treat pollen

(Meeus *et al.* 2014a; Graystock *et al.* 2016a; Simone-Finstrom *et al.* 2018; Álvarez Hidalgo *et al.* 2020).

Because there was a correlation between virus detection in pollen samples and virus abundance at the site level for each virus, pollen testing has the potential to be used as a non-lethal surveillance method for detecting viruses at the site level instead of sampling from individual hives. If pollen samples are additionally pooled, their predictive power of site-level viral profiles could be more accurate. This surveillance technique could be used in two ways, both requiring placing a pollen trap on a honey bee hive for a short amount of time prior to pollen collection. Surveillance hives could be placed at sites to evaluate the prevalence of viruses already present, either from nearby honey bees from other beekeepers, or potentially from wild bees. This could also serve to evaluate virus prevalence at a larger scale across many sites and help predict emerging virus issues. Alternatively, pollen could be collected from a hive at a site with honey bees already present, as a screening method for beekeepers to detect problematic bee yards that may require further *Varroa* surveillance and treatment.

Honey bee regulations

Beekeeping laws and regulations rarely include mentions of, or restrictions related to bee viruses. Global trade regulations for each country incorporate recommendations from the *Terrestrial Animal Health Code (Terrestrial Code)* published by the World Organisation for Animal Health (WOAH, previously IOE), which is followed by countries that are a part of the World Trade Organization (WTO) (Matheson 2000, Mutinelli 2011, World Organisation for Animal Health 2024). The *Terrestrial Code* does not include any recommendations for honey bee viruses, so they are generally not considered when making decisions on trading honey bees or their products with other countries (Mutinelli 2011; World Organisation for Animal Health

2024). This allows new virus variants to spread worldwide, potentially harming both honey bees and wild bee species. Within Canada, beekeeping regulations were recently reviewed by Leung and Reid (2025), and it is noted that most provinces/territories do not identify viruses in their provincial and territorial statutes and regulations. Manitoba happens to be one of the few provinces/territories that does identify viruses in its regulations (under the Diseases of Bees Designation Regulation, 2024), but there are no regulations on reporting virus detections (under the Reportable Diseases Regulation, 2007) (Leung and Reid 2025). In Canada, due to the cold climate limiting queen breeding and elevated overwintering colony losses over the last two decades, importing honey bees is currently necessary to support the beekeeping industry (Bixby *et al.* 2020, 2021; Claing *et al.* 2025). This unfortunately poses a risk of importing pathogens, so regulating this delicate system is important to ensure imported bees do not add additional stress to the beekeeping industry or negatively impact other susceptible pollinators. Monitoring virus infections, especially outbreaks of less commonly found viruses and emerging virulent strains, could be important both for beekeepers and for the continued monitoring of spillover into wild bees. Additionally, although common viruses like BQCV were widespread in honey bees and wild bees, monitoring efforts of this virus especially in wild bees could still be useful, as BQCV could be used as a marker of contact between honey bees and wild bees in future pathogen spillover research.

Highlighting the complexity of disease management in the beekeeping industry, an emerging pest of honey bees, *Tropilaelaps mercedesae*, an ectoparasitic mite (like *Varroa*), has driven some Canadian beekeepers to advocate for a ban on colony imports from outside of North America, and the removal of an import ban previously placed on mainland USA (Paradis and Awram 2025). The import ban has been in place since 1987 due to concerns over Africanized

bees and various honey bee pests, including acaricide resistant *Varroa* mites (Canadian Food Inspection Agency 2024). The original hosts of *Tropilaelaps* are the giant honey bees, but a host switch exposed *A. mellifera* to this threat (Chantawannakul *et al.* 2016). There is evidence that this mite may also be a competent vector of DWV and potentially ABPV (Dainat *et al.* 2009; Khongphinitbunjong *et al.* 2016; Chanpanitkitchote *et al.* 2018). While many studies have focused on the role *Varroa* plays in virus transmission between honey bees, virus transmission research focused on *Tropilaelaps* is still limited. Further virus research is needed to fully understand the threat that *Tropilaelaps* poses in comparison to other well-known pathogens, especially because *Varroa* has been shown to drive viral dynamics in both the honey bees they parasitize, and sympatric bumble bees populations (Manley *et al.* 2019). It is also important that regulations in place are supported by a thorough understanding of each pathogen, and that they reflect what is going to best support both the beekeeping industry and wild bee health.

Future Directions

Phylogenetics and sequencing

From our sequencing and phylogenetic data, we hypothesized that what we are observing is dead-end spillover and not true host expansion due to the lack of diversification we observed in wild bees (McKeown *et al.* 2025). This is consistent with most other phylogenetic studies, but with our limited number of samples we do not have enough data to rule out that true host expansion is not occurring. To make this determination, many virus sequences from the same wild bee species would be required to determine whether viruses from any single species form a cluster. Although sequences from wild bee species were intermixed with those from *A. mellifera* within trees, the only virus with multiple sequences from the same wild bee species was SBV, with two sequences from *B. borealis* which did not cluster (Figure 9). This is not enough

evidence to draw conclusions, so future studies similar to McKeown *et al.* (2025), which focused on the viromes of *B. impatiens* and *A. mellifera* over the course of three years, should be performed across a greater range of wild bee species.

Our phylogenetic analysis was only performed on select samples, primarily to confirm the identity of the virus, and on only a short section of each viral genome. While other studies have done more thorough phylogenetic analyses on these viruses, there is still much that can be done. Analyses to determine the evolutionary origins of bee viruses could potentially be useful, as the origins of many viruses are unclear. This knowledge could help us understand future risks associated with bee viruses and even provide a model for emerging diseases. Viruses like BQCV and SBPV appear to be relatively stable in terms of phylogenetic diversity and variants, but DWV-B has recently undergone a rapid expansion since it began its association with *Varroa* mites (Manley *et al.* 2019, 2020). This instability could have implications for the development of new, virulent variants and further host expansions.

More information on the origins of viruses, especially if the ancestral host is not honey bees, could also potentially inform regulations. Analyses by Manley *et al.* (2019, 2020) identified *A. mellifera* as the ancestral host of DWV-B and BQCV, but *Bombus pascuorum* (Scopoli, 1763) as the ancestral host of SBPV, although these studies only compared *A. mellifera* to three *Bombus* species. A phylogeographic analysis by Hasegawa *et al.* (2023) found that DWV-A likely originated in Asia, potentially in *A. cerana*, and spilled over to *A. mellifera* along with *Varroa* mites. On the other hand, they found that DWV-B appears to have originated in the Middle East, and the history of its emergence is less clear and would benefit from further study (Hasegawa *et al.* 2023). Ancestral virus hosts could be more susceptible to viruses and act as reservoirs, harbouring high viral loads and posing a risk to honey bees living in the same area.

Additionally, although in most studies, including ours, honey bees are found to be the species with the highest virus prevalence and intensity, there are some notable exceptions. McMahon *et al.* (2015) found that the species with the greatest SBPV prevalence in their study was *Bombus hortorum* (Linnaeus, 1761), and ABPV had a higher prevalence in *Bombus lapidarius* (Linnaeus, 1758) compared to *A. mellifera*. In an interesting case, de Souza *et al.* (2019) found that DWV loads were similar between honey bees and *Melipona subnitida* Ducke, 1911, a stingless bee, in mainland Brazil, but on islands where both species were introduced about 30 years prior DWV loads were greater in *M. subnitida* compared to honey bees. In cases like these, where an ancestral host has been identified or a bee species appears to be particularly susceptible to a virus, special restrictions may be useful to protect both the health of the wild bee species and the health of honey bees if contact poses a spillover risk. This spillover risk includes the potential to harm affected wild bee species, the further development of new virus strains as they adapt in wild bee hosts, and higher virus titres in susceptible bees, where wild bees may act as reservoir hosts and the virus could spill back to honey bees.

DWV-A/B recombinants have been frequently reported in honey bees and are becoming increasingly dominant (Hesketh-Best *et al.* 2024; Dobelmann and Wilfert 2025; Sircoulomb *et al.* 2025). Our study only used a small portion of the DWV genome for quantification and sequencing. Without sequencing multiple areas of the genome, differentiating DWV-A and B from recombinants of the two master variants would not be possible, so some of our DWV detections could potentially be DWV-A/B recombinants. This information would be useful in detecting emerging strains that could have a higher virulence. For example, we found a single DWV-B sample in a honey bee (LR1HB4) with a high number of gene copies (1.5×10^{10} gene copies/ μ g RNA) in combination with a peak melt temperature (79.5°C) that differed from all

other samples. Although this sample clustered with the expected group (DWV-B vs DWV-A) when sequenced (Figure S2), sequencing more portions of the genome could give additional context and determine if this is a recombinant, or an uncommon or emerging DWV-B variant. This context can help researchers be certain of which strains are impacting bee populations while directing future pathogenicity studies.

Future field study designs

In this study, samples were only collected for one year, which is consistent with similar studies on this subject (Fürst *et al.* 2014; McMahon *et al.* 2015; Alger *et al.* 2019a; Tiritelli *et al.* 2024; Maurer *et al.* 2024). Multi-year studies on virus spillover to wild bees are lacking and understanding how viruses persist in wild bee populations and vary between years would add context to some results we observed. Interestingly, at one site (IS) in the first sampling round there was no DWV detected in honey bees, but nine of ten wild bees had detectable DWV-B, and four had DWV-A. By the second sampling round at the same site, no DWV was detected in honey bees or wild bees. It is possible that this DWV was present in wild bee populations at this site as a result of the previous year's virus abundance at the site, but without data from the previous year we cannot be certain. At another site (RR), where no honey bees have been kept since 2021 (when they were situated there for an experiment, see Wizenberg *et al.* 2024), no honey bees were observed during any sampling rounds. Still, wild bees were found to be positive for BQCV (four *B. vagans*, one *A. terminalis*) and SBV (one *B. vagans*, one *A. terminalis*) in the third sampling round despite the lack of honey bees present. It is unknown if viruses persisted in wild bee populations since 2021, or if there have been hives present nearby since 2021. If samples were collected over multiple years from the same sites, tracking these patterns of virus spillover and persistence in wild bee populations could be more accurate. A multi-year study

could also evaluate factors involved with spillover over time and potentially allow for the prediction of increased virus transfer or spillover in honey bee and wild bee populations based on the previous year's conditions. This could be useful in decisions regarding disease treatments in honey bee colonies.

We did not observe any effect of seasonality on DWV-B detections in wild bees, but DWV-B is known to peak in the fall (Traynor *et al.* 2016; Molinatto *et al.* 2025), which is after our sampling was completed. A longer sampling period, starting earlier and ending later, could reveal seasonal viral dynamics that we did not observe in this study, including the potential increase of DWV-B prevalence in the fall. Sampling earlier, combined with a multi-year study, could also help us evaluate what the prevalence of viruses are in wild bees in early spring before they have had the chance to acquire viruses from the environment that year. It would be useful to understand how viruses persist in wild bees over the winter, and how this varies based on a bee's overwintering life stage.

Although we did not detect any effect of virus abundance in honey bees on wild bee diversity, this effect would be difficult to detect with our study design. For most factors, 20 sites were sufficient for their evaluation, but both with our sampling regime and analysis, as all transect samples were combined to calculate diversity, more sites to evaluate wild bee diversity would have been beneficial. Additionally, using multiple sampling techniques to evaluate wild bee diversity would give a more accurate measure, as there were often few samples caught in transects, especially if flowers in the areas were limited (Prendergast *et al.* 2020; Pei *et al.* 2022). Sampling techniques such as bee bowls could make up for this deficit, especially since they may catch more bees when there are few desirable flowers and favour small bees, while net sampling can be biased towards larger, more obvious bees foraging on flowers (Prendergast *et al.* 2020).

For future studies on wild bee diversity, we also recommend using sites with consistent honey bee presence and disease management strategies over time. Sites used in our study were not consistently used as honey bee yards, and some sites that had many hives present in previous years (DM, BN) were not used as bee yards this year and were included as lower honey bee density sites. A study where the honey bees at a site are consistently present and managed would better evaluate the long-term effects of viruses on wild bee diversity, or vice versa. The perfect study would consider beekeeping history and historical virus prevalence in beekeeping operations and at sites, combined with a multi-year full season assessment. Although the perfect study is likely impossible given the many dynamics in this system, these factors are worth considering in future monitoring programs, especially when working directly with beekeepers.

Pathogenicity studies

Knowing which factors influence pathogen spillover is useful, but this knowledge is limited without a proper understanding of the infectivity and pathogenicity of viruses in non-*Apis* bees. In our study, we only report virus detections, including the quantification of viral gene copies. This is not a confirmation of infection, which requires at least the detection of the viral negative strand to confirm positive-sense virus replication in the host (Radzevičiūtė *et al.* 2017; Warncke and Knudsen 2022). It should also be noted that because bees were sampled while foraging, it would be unlikely to capture highly infected individuals with symptoms (Fürst *et al.* 2014). Flight could be impacted in symptomatic individuals, as seen in honey bees and bumble bees infected with DWV (Genersch *et al.* 2006; de Miranda and Genersch 2010), and we did not observe any bees with these characteristic wing deformities. This study is solely focused on factors influencing the acquisition of viral gene copies by wild bees.

Most pathogenicity studies performed on non-*Apis* bees have been done on *Bombus* (Genersch *et al.* 2006; Fürst *et al.* 2014; Meeus *et al.* 2014a; Piot *et al.* 2015; Graystock *et al.* 2016b; Manley *et al.* 2017; Streicher *et al.* 2023), with the exceptions of one study using *Melipona beecheii* (Fleites-Ayil *et al.* 2023), a study using *Megachile rotundata* and *Colletes inaequalis* (Dolezal *et al.* 2016), a study just focused on *M. rotundata* (Singh 2011), and a study on *Osmia bicornis* (Schauer *et al.* 2023). The studies on *Bombus*, *M. beecheii*, and the Singh (2011) study on *M. rotundata* detected adverse effects of viruses on inoculated bees. More species need to be tested so we can better evaluate the risks viruses pose to wild bee health, and to commercial non-*Apis* species. More studies comparing the effects of viruses on larvae and adults would also be beneficial, as some viruses like SBV are best known for their effects in young larvae (Chen and Siede 2007), so symptoms may not be observed if only adults are inoculated.

Since other stressors including coinfections, either with other viruses (Remnant *et al.* 2019; Durand 2024) or non-viral pathogens (Di Prisco *et al.* 2016; Gajda *et al.* 2021), nutritional stress (DeGrandi-Hoffman *et al.* 2010; Dolezal *et al.* 2019; Zhang *et al.* 2020), and pesticide exposure (Doublet *et al.* 2015; Fine *et al.* 2017; Coulon *et al.* 2018) have been shown to affect the pathogenicity of certain viruses in honey bees, these are also assays that should be performed on other bee species to evaluate their effects. For example, because in honey bees *V. apis* and *ceranae* appear to increase mortality in combination with BQCV infections (Bailey *et al.* 1983; Gajda *et al.* 2021), studying interactions between *Vairimorpha bombi* (Fantham and Porter, 1914), a parasite of *Bombus* (Otti and Schmid-Hempel 2007; Cameron *et al.* 2011), and BQCV, which had a prevalence of 73.7% in our tested bumble bees, would be a worthwhile study. The few studies that have tested the effect of viruses on stressed bumble bees, examining nutritional

stress (Manley *et al.* 2017) and the neogregarine *Apicystis bombi* (Liu, Macfarlane, & Pengelly, 1974) (Graystock *et al.* 2016b), found that the combination of virus infection and stressful conditions could increase bee mortality. Additionally, many pathogenicity studies have focused on only how these viruses impact mortality, which while important, is likely not the only symptom of these viruses. In honey bees, viruses have been found to impact orientation, learning, and foraging, so research on the non-lethal effects of viruses is also needed in other bee species (Bailey and Fernando 1972; Anderson and Giacon 1992a; Iqbal and Mueller 2007; Li *et al.* 2013; Wells *et al.* 2016; Retschnig *et al.* 2019). Even in honey bees, studies on non-lethal effects of viruses are limited, whereas this has been more extensively studied for pesticides (Decourtye *et al.* 2005; Belzunces *et al.* 2012; Williamson and Wright 2013; Frost *et al.* 2013; Siviter *et al.* 2018). In general, testing the non-lethal effects of viruses is an understudied yet crucial area of research for understanding bee viruses.

SUMMARY AND CONCLUSION

This study provides evidence that pathogen spillover from honey bees to wild bees is occurring, and a variety of factors are influencing this virus transfer. With greater virus abundance in sympatric honey bees, wild bees were more likely to be detected with BQCV, SBV, and DWV-B. Greater agricultural intensity also increased the transfer risk of BQCV and SBV to wild bees and was additionally associated with decreased wild bee diversity. Potentially linked to this is the effect of floral abundance on transfer risk; canola bloom was highly associated with floral abundance, and DWV-B was more likely to be detected in wild bees when floral abundance was high. Additionally, wild bee species more closely related to honey bees were more likely to harbour BQCV and SBV compared to those more distantly related. DWV-B appears to be a more generalist virus, as relatedness did not affect its presence in wild bees. Seasonality also affected virus transfer risk; wild bees collected later in the summer were more likely to harbour detectable BQCV and SBV. Kleptoparasitic bees also appear to be at a greater risk of virus transfer and were found more likely to harbour SBV compared to eusocial and solitary bees. As expected, if virus transmission is occurring between honey bees and wild bees, viruses showed no evidence of clustering based on host species, and any phylogenetic patterns present appeared to be based on geographic location. In this study, many variables measured are proxies, so more controlled experiments to test how various factors impact virus transmission in isolation is necessary. In addition, infection experiments testing the lethal and non-lethal effects of viruses on a wide range of bee species is essential to truly understand the impact that viruses have on wild bees, as so little is known on this topic outside of *Bombus*.

It was also found that bumble bees are collecting pollen with viruses present, and these viruses appear to be deposited by other pollinators present at the site. We found bees that

collected pollen with SBV and DWV-B present, while they themselves did not have any detectable corresponding virus. Bees were also found with BQCV, SBV, and DWV-B present, while the pollen they collected was virus-free. It appears likely that pollen and shared floral resources are important in virus transfer, and that DWV-B and SBV are primarily transferred to pollen through the fecal-oral route or another pathway associated with pollinator traffic on flowers, as opposed to through saliva or nectar regurgitation. BQCV appears to differ in its ecology when compared to DWV-B and SBV, as the virus found in the individual bumble bee that collected a pollen sample was a better predictor of virus presence in pollen than the overall virus abundance at a site. It is possible that BQCV is more easily transmitted compared to other viruses, and that virus transfer through saliva or regurgitation may play a more prominent role in its spread. More well-designed, comparative transmission experiments in the laboratory are required to properly assess these potential differences in viral ecology.

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APPENDIX

Table S1: Annual Crop Inventory land use types associated with each code, and the class given to each land use type (Agriculture and Agri-Food Canada 2024). Codes are represented as colours in the plots in Figure 1.

Code	Label	Class
20	Water	semi-natural
30	Exposed land/barren	urban
34	Urban/developed	urban
35	Greenhouses	urban
50	Shrubland	semi-natural
80	Wetland	semi-natural
110	Grassland	semi-natural
122	Pasture/forages	agriculture
130	Too wet to be seeded	agriculture
133	Barley	agriculture
136	Oats	agriculture
137	Rye	agriculture
145	Winter wheat	agriculture
146	Spring wheat	agriculture
147	Corn	agriculture
153	Canola/rapeseed	agriculture
158	Soybeans	agriculture
162	Peas	agriculture
167	Beans	agriculture
177	Potatoes	agriculture
210	Coniferous	semi-natural
220	Broadleaf	semi-natural
230	Mixedwood	semi-natural

Table S2: Detailed land use in the 1km surrounding each site. All values are in percent of land use. Land use data was acquired from the 2024 Annual Crop Inventory (Agriculture and Agri-Food Canada 2024).

Site	Water	Exposed land/ barren	Urban/ developed	Shrubland	Wetland	Grassland	Spring wheat	Broadleaf	Mixedwood	Pasture/ forages	Too wet to be seeded	Corn	Canola/ rapeseed	Soybeans	Coniferous	Barley	Oats	Peas	Greenhouses	Rye	Beans	Potatoes	Winter wheat
FW	4.79	11.53	58.04	1.35	2.35	11.56	0.32	10.04	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TG	0.17	2.18	4.05	2.27	0.75	40.83	5.57	37.58	0.06	2.30	0.34	1.03	2.27	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SW	0.72	3.67	33.78	0.20	0.23	3.50	0.80	13.26	0.06	0.00	0.00	0.00	0.92	42.82	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LS	0.00	2.26	4.01	1.58	0.14	25.63	0.74	36.54	0.14	0.00	1.63	5.30	12.63	7.13	0.03	0.11	2.12	0.00	0.00	0.00	0.00	0.00	0.00
BP	5.51	7.67	5.94	0.34	0.66	22.14	3.22	12.81	0.03	0.00	0.00	0.52	23.74	17.37	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LR	6.07	1.92	1.38	8.28	4.70	68.42	0.00	8.65	0.03	0.00	0.00	0.00	0.00	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SM	0.32	23.18	33.83	0.78	0.09	11.83	3.39	10.22	0.11	4.65	0.20	0.00	9.91	1.15	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MS	0.00	4.83	2.13	1.32	0.32	8.91	0.00	55.83	5.06	0.11	0.00	0.34	0.09	1.06	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RR	0.69	1.63	4.96	0.69	0.89	14.61	0.00	76.02	0.00	0.00	0.00	0.00	0.00	0.46	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SA	0.06	0.89	2.95	1.20	0.97	0.17	0.00	21.01	0.06	0.00	0.00	0.00	0.00	0.00	72.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PV	0.06	3.38	3.98	0.00	0.00	0.63	10.04	0.54	0.00	0.00	0.00	1.12	39.97	22.63	0.00	0.00	17.65	0.00	0.00	0.00	0.00	0.00	0.00
DW	0.03	1.64	3.56	0.03	0.00	0.66	44.72	0.92	0.00	0.00	0.00	0.00	44.69	3.59	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00
IN	0.03	0.17	1.81	1.89	0.23	1.15	28.28	6.57	0.00	0.00	0.00	1.12	45.27	0.66	0.00	12.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00
IS	0.17	0.20	1.98	3.30	0.37	3.30	11.58	3.24	0.00	0.00	0.00	0.00	49.64	3.55	0.00	13.44	9.23	0.00	0.00	0.00	0.00	0.00	0.00
DM	0.43	4.70	29.04	0.06	0.66	0.37	11.11	6.59	0.00	0.00	0.00	3.04	17.30	2.29	0.11	0.00	24.31	0.00	0.00	0.00	0.00	0.00	0.00
BN	0.06	0.34	3.44	0.03	0.03	0.43	10.74	0.00	0.00	0.00	0.00	0.00	52.12	20.63	0.00	0.00	11.83	0.34	0.00	0.00	0.00	0.00	0.00
CM	0.40	4.51	23.70	3.21	0.09	2.27	5.08	17.30	0.32	0.00	0.00	0.86	11.68	12.91	0.00	0.95	2.09	0.00	2.96	0.40	11.16	0.11	0.00
RT	0.11	0.06	0.00	0.17	0.26	0.60	23.17	0.06	0.00	1.12	0.00	0.00	46.28	22.65	0.00	0.11	0.00	0.00	0.00	3.67	0.00	0.00	1.75
GL	0.06	0.11	3.01	0.00	0.00	0.32	41.41	0.98	0.00	0.00	0.00	0.00	21.59	32.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PS	0.09	3.18	4.10	0.03	0.03	0.69	19.50	0.37	0.00	0.00	0.00	2.35	43.33	19.56	0.03	0.00	6.76	0.00	0.00	0.00	0.00	0.00	0.00

Table S3: Sequencing results for select samples, confirming their identity. The table includes the sample ID, host bee species, target virus, peak melt curve temperature, the identity of the top match(es) using the NCBI BLAST tool, and the percent identity of the top match.

Sample ID	Host species	Virus	Melt temp. (°C)	Identity of top match(es)	Top match %
SM2HB0	<i>Apis mellifera</i>	BQCV	81.0	BQCV	100%
IN2WB7	<i>Heriades carinata</i>	BQCV	81.0	BQCV	100%
BP2WB8	<i>Hylaeus affinis</i>	BQCV	81.0	BQCV	100%
IS2WB2	<i>Nomada composita</i>	BQCV	81.0	BQCV	100%
PS1WB7	<i>Lasioglossum perpunctatum</i>	BQCV	81.0	BQCV	99.65%
DM2HB2	<i>Apis mellifera</i>	SBV	81.5	SBV	98.35%
DW3WB3	<i>Bombus rufocinctus</i>	SBV	81.5	SBV	98.69%
PV3WB5	<i>Megachile inermis</i>	SBV	81.5	SBV	99.02%
PS2HB9	<i>Apis mellifera</i>	SBV	82.0	SBV	100%
IN3WB7	<i>Dufourea marginata</i>	SBV	82.0	SBV	99.01%
LS2WB3	<i>Heriades carinata</i>	SBV	82.0	SBV	100%
PV1HB6	<i>Apis mellifera</i>	SBV	82.5	SBV	98.70%
PS3WB4	<i>Bombus borealis</i>	SBV	82.5	SBV	98.38%
PS1WB5	<i>Stelis lateralis</i>	SBV	82.5	SBV	98.34%
BP2WB0	<i>Bombus borealis</i>	SBV	83.5	SBV	98.76%
PV3WB8	<i>Megachile inermis</i>	SBV	83.5	<i>Bombus vosnesenskii</i>	98.70%
RT1WB1	<i>Lasioglossum c.f. semicaeruleum</i>	SBV	84.0	<i>Megachile rotundata</i>	92.89%
SM1HB0	<i>Apis mellifera</i>	DWV-B	78.0	<i>Lasioglossum baleicum</i>	92.83%
IS1WB5	<i>Andrena wilkella</i>	DWV-B	78.0	DWV-B and DWV	99.15%
LR1HB3	<i>Apis mellifera</i>	DWV-B	78.5	DWV-B and DWV	100%
IS1WB4	<i>Bombus borealis</i>	DWV-B	78.5	DWV-B	100%
IS1WB7	<i>Halictus rubicundus</i>	DWV-B	78.5	DWV-B and DWV	100%
LR3HB8	<i>Apis mellifera</i>	DWV-B	79.0	DWV-B and DWV	99.29%
LR1HB4	<i>Apis mellifera</i>	DWV-B	79.5	DWV-B and DWV	98.44%
LR1HB9	<i>Apis mellifera</i>	DWV-A	80.0	DWV	99.27%
SM3HB8	<i>Apis mellifera</i>	DWV-A	80.5	DWV	99.10%
IS1WB5	<i>Andrena wilkella</i>	DWV-A	80.5	DWV-A and DWV	100%
FW2HB9	<i>Apis mellifera</i>	DWV-A	81.0	DWV-A and DWV	100%
GL2HB4	<i>Apis mellifera</i>	DWV-A	81.5	DWV	100%
DM2HB2	<i>Apis mellifera</i>	IAPV	81.5	IAPV	98.03%
GL2HB5	<i>Apis mellifera</i>	IAPV	82.0	IAPV	100%
RT3WB7	<i>Andrena thaspia</i>	IAPV	82.0	IAPV	100%

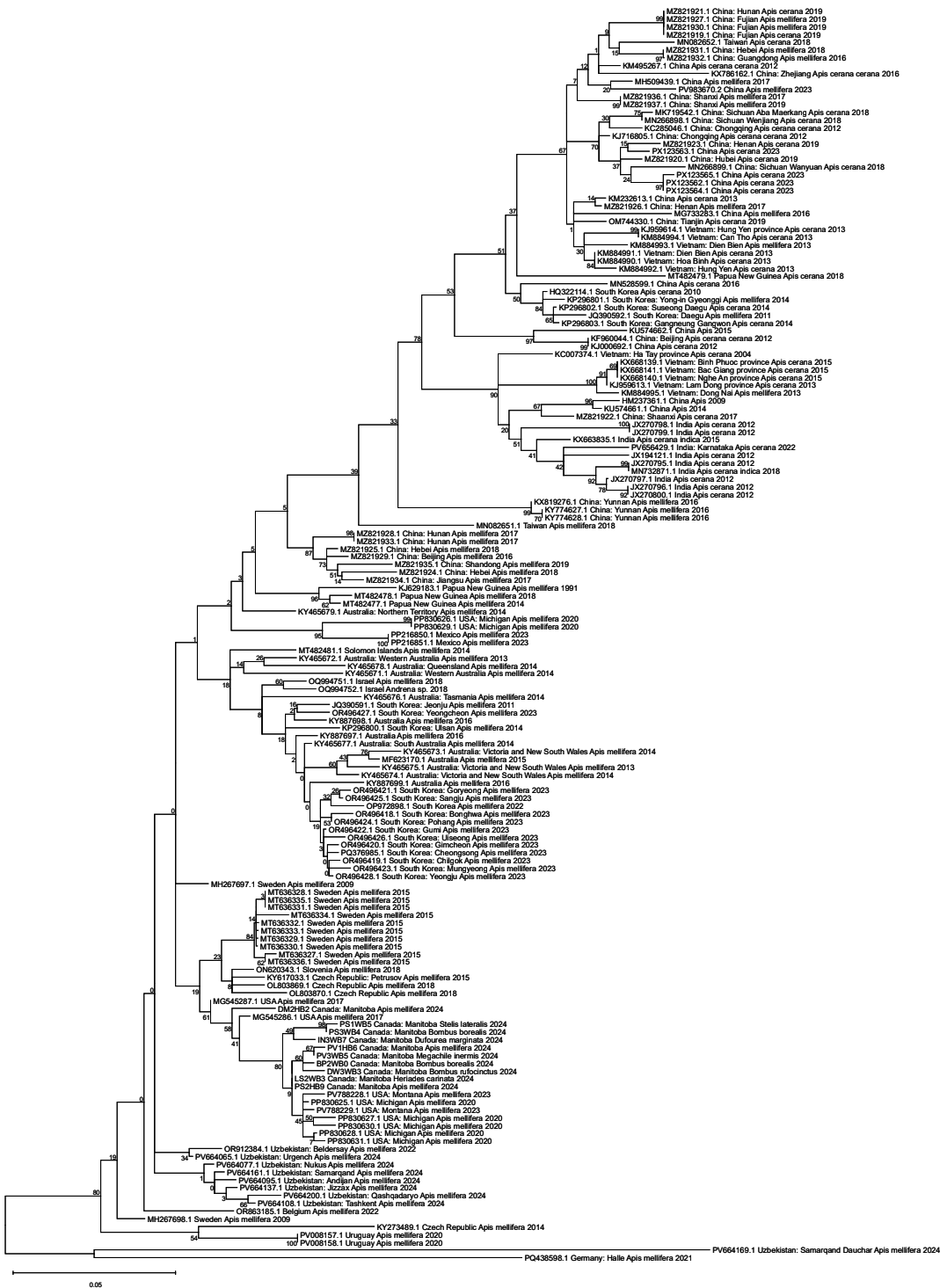


Figure S1: Evolutionary analysis of SBV in study samples and those available from GenBank aligning with the study samples, conducted and visualized in MEGA12 (Kumar *et al.* 2024). The phylogeny was constructed using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993) of nucleotide substitutions using MEGA12 (Kumar *et al.* 2024). The displayed tree is the one with the highest log likelihood (-4322.87) and is unrooted. Bootstrap support (1000 replicates) is shown for all nodes (Felsenstein 1985). The analysis comprised of 161 sequences and 306 nucleotide positions.

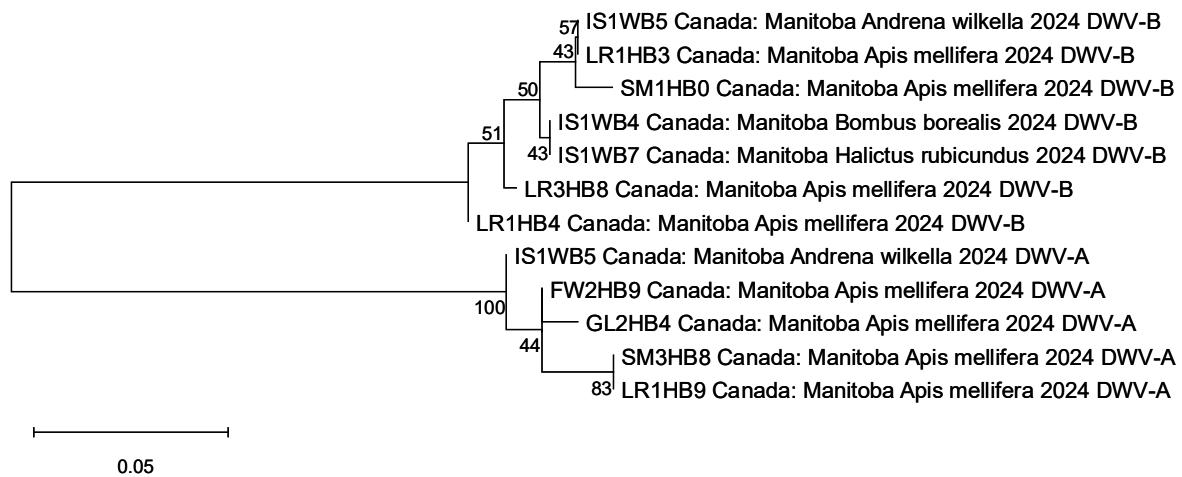


Figure S2: Evolutionary analysis of DWV in study samples, conducted and visualized in MEGA12 (Kumar *et al.* 2024). The phylogeny was constructed using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993) of nucleotide substitutions using MEGA12 (Kumar *et al.* 2024). The displayed tree is the one with the highest log likelihood (-275.34) and is unrooted. Bootstrap support (1000 replicates) is shown for all nodes (Felsenstein 1985). The analysis comprised of 12 sequences and 109 nucleotide positions.

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