Viruses of Mason Bees in the Sierra Nevada

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ABSTRACT

Bees play a vital role pollinating a wide variety of flowering plants, including commercial crops. The movement of honey bee colonies for the purpose of commercial pollination has distributed viruses across the globe. These viruses have the potential to spillover to native bee populations, although these dynamics are often poorly understood for many different types of bees. In this study I aimed to understand the viruses of a native bee genus, *Osmia*, in the Tahoe National Forest region of the Sierra Nevada. Honey bee hives were experimentally placed at three sites and bees were sampled at sites with and without apiaries before and after honey bee arrival. Taxonomic experts identified thirty-one species of mason bee, representing over a quarter of North American *Osmia* diversity. Bee RNA was processed and pooled samples were sequenced using a metatranscriptomic approach. From this data we identified three viruses: Deformed wing virus (DWV-B), Chronic Bee Paralysis Virus (CBPV), and Andrena-associated Bee Virus (AnBV-1). This is the first time that AnBV-1 has been found in *Osmia* bees to our knowledge. Nine individuals were infected with DWV-B and 5 were infected with CBPV. In CBPV, an 892.54% increase of prevalence was found to be associated with the arrival of honey bee colonies to the study area. A baseline understanding of the viruses infecting mason bees in this region provides a starting point for future analysis of the impacts that commercial honey bee colonies have on native bees.

KEYWORDS

Mason bees (*Osmia)*, Disease Ecology, Native Bee Viruses, Deformed Wing Virus, Chronic Bee Paralysis Virus

INTRODUCTION

Pollination is an important ecosystem service for human crop production, responsible for 91 out of 107 of the world's leading fruit and seed-bearing crop species grown for consumption (Klein et al. 2007). Insect-mediated pollination of crops in the United States is estimated to be worth 31.8 to 36.2 billion USD, with California's pollination alone valued at 6.3 to 9.4 billion USD (Jordan et al. 2021). Bees as a clade account for the majority of insect pollination visits to crops (Rader et al. 2016). Although animal-mediated pollination services face a wide variety of threats, understanding pathogens is of particular importance. From 2017 through 2022 beekeepers in the United States reported that nearly half of managed honey bee (*Apis mellifera*) colonies were lost annually, with the mite *Varroa destructor* and its associated viruses being one of the most consistently reported causes of loss (Bruckner et al. 2023, Aurell et al. 2023). Understanding these viruses is therefore crucial to protecting pollination services worldwide.

Aside from *V. destructor*, the environment plays an important role in transmitting viruses between species through horizontal transmission. For example, Black Queen Cell Virus (BQCV), Deformed Wing Virus (DWV), and Sac Brood Virus (SBV) samples collected from food sources such as pollen pellets were able to infect healthy honey bees (Singh et al. 2010). And when infected bees visit flowers, they can deposit their viruses, which can infect other bees (Mazzei et al. 2014, Burnham et al. 2021). Additionally, flowers that are visited for longer periods of time are more susceptible to contamination (Alger et al. 2019). Although honey bees and their associated viruses are well studied, they are non-native to the Americas, being introduced by European colonizers in the 17th century. Only a small fraction of academic literature focuses on solitary bees, despite the group containing more taxa than their social counterparts (Tehel et al. 2016). California alone is home to over 1,600 species of native bees, and their population dynamics are largely unknown. The data we do have is mostly for bumblebees, and there we see many critical species experiencing severe declines, which has prompted the California Department of Fish and Wildlife to list 4 species of bumble bees as endangered under the California Endangered Species Act as of 2022 (Jepsen 2022). Solitary bees as a whole are understudied, and accordingly, understanding the wide variety of solitary bees and their viruses is important to address conservation concerns.

Mason bees (*Osmia*) are an important pollinator for many flowering plants and have many species native to North America. In light of threats to commercial honey bees, many farms have

turned to supplementing honey bee pollination with mason bees, including the species *O. bicornis*, *O. cornifrons*, *O. cornuta*, and *O. lignaria* (Osterman et al. 2021). Mason bees are solitary cavity-nesting bees that construct brood chambers in the nest using mud. Female mason bees place pellets of pollen for their offspring in each chamber, and then lay an egg on or near each pellet. Due to their distinct life histories, it is largely unknown how the virus dynamics of mason bees relate to honey bees. Some viruses have been shown to spillover from honey bees and actively replicate, such as BQCV in *O. bicornis* (Radzevičiūtė et al. 2017). Alternatively, DWV has been shown to not replicate in the same species, despite being present (Schauer et al. 2023). Other honey bee viruses, like SBV, Israeli acute paralysis virus (IAPV), Chronic Bee Paralysis Virus (CBPV) and Kashmir bee virus (KBV) have been detected in non-*Osmia* bee taxa and other non-bee insects (Levitt et al. 2013), but are still being explored in *Osmia*. As research expands more mason bee viruses are being discovered. For example, *Osmia*-associated bee chuvirus (OABV) was recently discovered in *O. taurus* bees from Fukushima, Japan (Takemae et al. 2023). To address conservation concerns, it is important to establish a baseline understanding of mason bee viruses by studying wild populations.

In this study I investigate the viruses that are infecting mason bees across nine sites in the Sierra Nevada mountains of California, a field system with low honey bee abundance, allowing for sampling of the native bee community before and after honey bees are experimentally moved to these sites for wildflower honey production. In the summer of 2021, PhD candidate Nina Sokolov conducted temporal sampling of foraging honey, bumble, and mason bees across sites with and without honey bee apiaries. In order to gain a baseline understanding of mason bee viruses, I ask what viruses are present in this study system and what species of mason bee might they be infecting, does prevalence change upon honey bee arrival, and does the local honey bee abundance or the proximity to an apiary affect virus prevalence? I expect to find a wide diversity of mason bees with few honey bee viruses due to the solitary nature of mason bees lessening intraspecies transmission compared to honey bees. Furthermore, I expect to find that prevalence will be higher at sites closer to apiaries and with higher honey bee abundance because honey bee presence increases the spillover pressure of viruses in mason bees.

METHODS

Osmia Life History

The genus *Osmia* (Family: Megachilidae) consists of solitary bees that build nests in cavities with mud. *Osmia* have a lifespan of roughly one year, most of which is spent in the nest. The larval stage begins after the eggs hatch in the spring, at which time the larvae consume a pollen provision left by their mother. After completion of the five larval instars, the larvae form a cocoon and develop into pupae and then adults by autumn. The adult overwinters in the cocoon, which is rare among Megachilidae (Bosch et al. 2001). In the late winter or early spring, most *Osmia* species emerge from the nest. Males typically emerge first, where they wait for a mate and die shortly after mating. Females construct nests and forage for pollen. Unlike honey bees, which often forage up to 6 km away from the colony, mason bees forage less than 1 km from the nest (Visscher and Seeley 1982, Zurbuchen et al. 2010). Females lay each egg on a pollen provision, with eggs being separated from each other in chambers partitioned by mud cell walls (Figure 1).

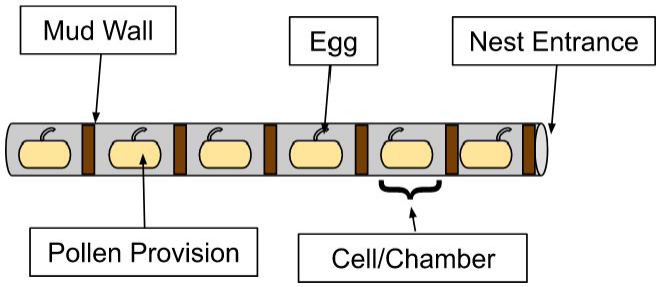

Figure 1. The organization of a generic *Osmia* nest cavity, fully provisioned.

Study Sites and Bee Collection

Nina Sokolov, a PhD candidate in UC Berkeley Boots' lab net-collected a total of 333 foraging *Osmia* specimens across nine sites varying in honey bee abundance in the Tahoe National Forest of the northern Sierra Nevada (Figure 2). Three treatment sites had apiaries experimentally set up which consisted of 25 managed honey bee hives being placed on private land within the Tahoe National Forest. The treatments were compared to control sites that were selected based on having a similar floral composition, while being as far away from the closest apiary as possible.

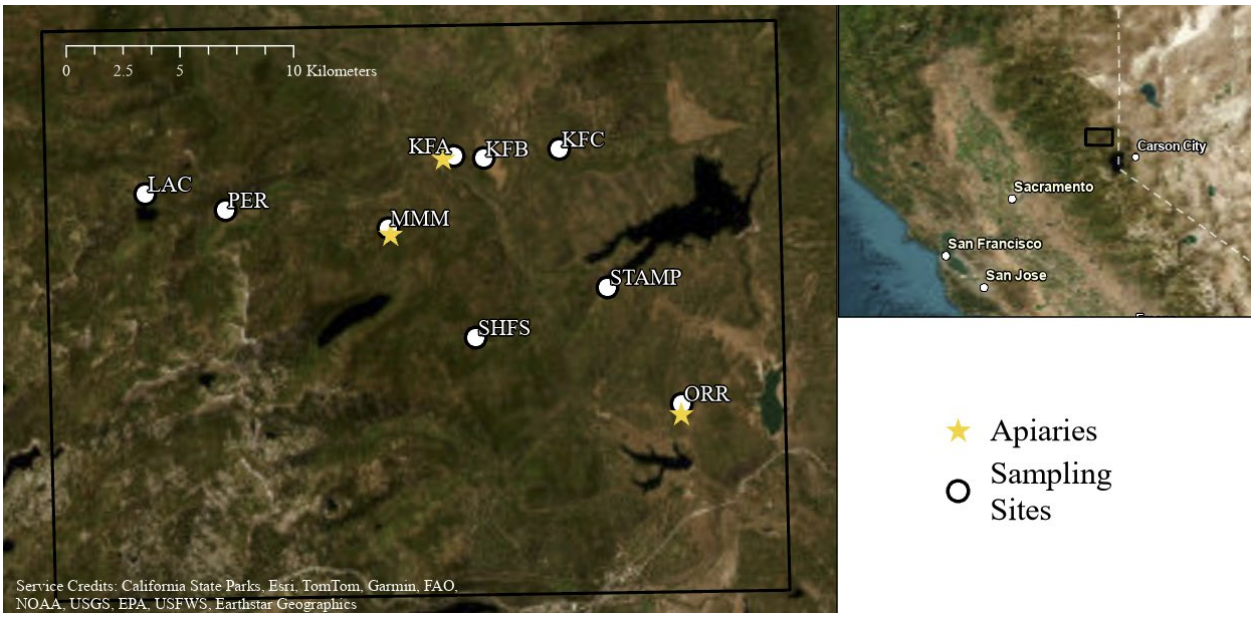

Figure 2. (Left) Distribution of sampling sites (white dots) and apiaries (yellow stars) in the Tahoe National Forest. (Right) Site location in reference to the Bay Area. Map was generated using ArcGIS Pro version 3.2.1.

To determine the species diversity differences, a bee diversity collection was created at each time point for each site. This involved sampling the greatest diversity of bees possible across different floral species within 20 person minutes. Bees were net collected from flowers before being placed into a jar with ethyl acetate for euthanization. These bees were then pinned and frozen for species identification and subsequent contribution to UC Berkeley's Essig Museum of Entomology. Dr. John Ascher (University of Singapore) and taxonomic exports at the USDA Logan Bee Lab, including Dr. Terry Griswold and Skyler Burrows identified the 187 pinned individuals. This pinned and identified reference collection would then have each individual species sequenced for the COI gene to provide a species reference DNA barcode, as most of these species were not yet in DNA barcoding databases. This would allow for any unidentified frozen bees to also be sequenced for this same gene, to facilitate DNA based identification, rather than morphological based, due to the exceedingly difficult taxonomic challenges of this genus. Honey bee abundance for each site and time point was determined by counting the number of individuals from the transect line and up to 2.5 meters on either side of a 100 m transect while at a predetermined pace through the densest patch of flowers. Therefore, sampling abundance was quantified at 500 m^2 (Gunnarsson and Federsel 2014).

Viral sampling occurred in 3 rounds per site across the summer of 2021 (Table 1); round 1 was before honey bee arrival, round 2 was immediately after arrival, and round 3 was several

weeks after arrival. Directly upon collection, Nina placed the mason bees on dry ice to be euthanized while preserving the viral RNA before being placed in a liquid nitrogen dewar in the field and subsequently stored at -80 degrees Celsius until they were ready for further processing.

Table 1. Sites and their respective number of mason bees collected for sampling at each time point.

Site	Time Point 1	Time Point 2	Time Point 3
KFA	5	5	3
KFB	5	3	0
KFC	10	10	5
LAC	0	5	0
MMM	13	5	6
ORR	9	3	0
PER	0	10	8
SHFS	11	11	9
STAMP	0	10	0

To gain a better understanding of the possible viruses in the study region, I purchased a total of 220 dormant *Osmia* from Foothill Bee Ranch (California, USA) for the purpose of RNA metatranscriptomics. One male and one female were collected by Foothill Bee Ranch from 10 *O. lignaria* and 10 *O. ribifloris* nests at five sites across the Sierra Nevada foothills in California, with an additional 10 male and 10 female *O. lignaria* collected from a sixth site.

Virus Sequencing

RNA Extraction

To prepare for RNA extraction and sequencing, I dissected each forager bee along the midsagittal plane from the head to the stinger on a bed of dry ice. I stored one half of the bee for backup, and I placed the other half in a 2.0 mL bead beating tube along with 1 mL of 0.5 mm zirconium beads and a single 3mm sterilized steel bead and stored them at -80 degrees Celsius until ready for RNA extraction. Additionally, one hind leg of each bee was saved for future DNA barcoding endeavors to identify any positive cases down to species. I did not dissect the bees from

Foothill Bee Ranch, rather I simply removed them from their cocoons and washed them with phosphate-buffered saline to wash off external fungus that had grown during shipment before placing them in a homogenizer tube with beads.

To remove the large host matter from the sample, Nina and I first added 750 mL of Trizol to each tube containing homogenizing beads and a bee. To break open the host cells, we placed the samples into a tissue homogenizer (FastPrep-24 5G bead beating grinder and lysis system) for three cycles of 15 seconds at 4 m/sec, with 30 seconds of rest in between cycles to keep the samples from heating enough to denature the viral RNA. We then placed the samples in a centrifuge for 1 minute to condense large host matter at the bottom of the tube. Next, we extracted the supernatant and placed it into a new tube, discarding the original tube with the leftover host matter.

To separate the RNA from the rest of the host matter, we added 75 µL of 1-bromo-3-chloropropane before shaking the samples gently for ten seconds and letting them rest. After five minutes we shook them once more and let them rest for another five minutes. Then, we placed the samples in a 4 degrees Celsius centrifuge at 14,000 rpm for 10 minutes. We then transferred the supernatant in 75 µL increments to a new tube, being careful not to pierce the lipid layer separating the suspended RNA from a liquid layer of contaminants, along with 375 µL of isopropanol. We discarded the old tube and let the new mixture rest for 7 minutes.

To force the RNA to precipitate at the bottom of the tube, we centrifuged the sample at 4 degrees Celsius and 14,000 rpm for another 10 minutes. After discarding the supernatant, we washed each RNA pellet with 750 µL of ethanol two times, discarded the final ethanol wash, and allowed the pellets to dry for 5 minutes before suspending them in 75 µL of water. We aliquoted 5 µL of the water-suspended RNA into strip caps for spectrophotometry, and an additional 25 µL into another set of strip caps for downstream analysis. To know which samples require cleaning, we put a drop of the 1 µL aliquots onto a Nanodrop ND-1000 to generate an absorbance spectrum. We characterized samples with absorbance spectrum 260/280 and 260/230 ratios of 2.0 (± 0.1) as clean and able to continue in downstream analysis.

Metatranscriptomics

We pooled two comparative RNA samples of 10 clean wild forager bees and 10 clean overwintering bees. To focus specifically on the RNA, any remaining DNA in these samples was

removed using Turbo DNAse Treatment (Thermo Fisher Scientific). Briefly, buffer and the Turbo DNase enzyme were added to the samples and incubated at 37 degrees for 30 minutes. Subsequently, the DNAse inactivation reagent was added and incubated at room temperature before being centrifuged and removed. Then, samples were cleaned of contaminants using an RNA Clean & Concentrator (Zymo). This involved adding a series of binding, prep and wash buffers to the sample columns and centrifuging repeatedly before eluting in clean nuclease free water. These cleaned RNA samples were sent to Novogene Inc. (Sacramento, California, USA) for library prep and subsequent RNA metatranscriptomic sequencing to quantify the entirety of the microbial community found in these pooled samples. We used the results of the shotgun sequencing to order primers for downstream analysis. Novogene conducted the bioinformatic analysis for these samples.

cDNA Synthesis and Analysis

We only synthesized the cDNA of the wild foraging bees, as most of the dormant bees from Foothill Bee Ranch were contaminated with *Ascosphaera* fungus, which made it difficult to obtain a clean enough sample of RNA to perform further analysis. We performed RT PCR to synthesize cDNA from the total RNA sample using MML-V Reverse Transcriptase (Promega). For each sample, 2 µg of RNA were added to nuclease free water along with random primers before being heated at 70 degrees Celsius for 5 minutes to melt secondary structures and bind the random primers to the template. Then the sample was placed directly onto ice before each sample was mixed with a mixture of buffer, dNTPs, and the MML-V enzyme before being incubated again at 37 degrees Celsius for 60 minutes. Using primers for the viruses identified in the metatranscriptomic analysis from Novogene Inc. we amplified the DWV-B, CBPV, and AnBV-1. We used GoTaq green master mix (Promega) and mixed 2 µl of template cDNA with 12.5 µl of master mix, 9.5 µl of nuclease free water, and 1 µl of forward and reverse viral primers. We also ran positive controls using the BeeCox1 gene primers that are being used as a specific DNA barcode for bees.

To identify the viruses in individual bee samples, we performed gel electrophoresis. We made 2% agarose gels stained with ethidium bromide and ran them at 120 v for 50 minutes before imaging using a Gel Doc Imager. Individual bees were scored as either 0 for no amplification or 1

if there was a fluorescent band that had the correct number of base pairs associated for each viral primer when compared to a 1 kb ladder. A score of 1 indicated evidence that the bee had the focal virus of interest.

Apiary Proximity

To analyze the relationship between honey bee presence and virus infection in mason bees, I first input apiary locations and mason bee collection events into ArcGIS Pro version 3.2.1 (Esri 2023). I then used the buffer tool to add a 1 km buffer to each collection event, representing the likely foraging range of mason bees and taking into consideration that the bees may have been several hundred meters from their nest when collected. Next, I classified sites with collection buffers that contained an apiary as IN, sites with collection buffers that are within 6 km of an apiary, representing the foraging range of honey bees, as NEAR, and sites with collection buffers further than 6 km from an apiary as FAR.

Statistical Analysis

To determine if virus prevalence differed between the three time points, I performed an analysis of variance (ANOVA). To assess the relationship between honey bee abundance and virus prevalence I performed a Pearson product-moment correlation analysis. For this analysis, I only included data from time points 2 and 3, because the honey bee colonies were not present during time point 1 and no wild honey bees were observed. To evaluate the relationship between apiary distance and virus prevalence, I performed an ANOVA. I also omitted time point 1 data from this analysis for both viruses as the apiaries were absent during this time, and time point 2 data was omitted because virus prevalence was zero at all sites except for one. All data analysis was performed using R version 4.3.2 (R Development Core Team 2023).

RESULTS

Bee and Virus Diversity

The taxonomic experts referenced above identified a total of 33 species of mason bee, including three unidentified, potentially undescribed, species (Table 2).

Table 2. *Osmia* species identified by experts at the USDA Logan Bee Lab and the University of Singapore.

Genus	Species	Genus	Species
Osmia	*"Acanthosmioides" sp.*	*Osmia*	*lanei*
Osmia	*albolateralis*	*Osmia*	*lignaria propinqua*
Osmia	*atrocyanea*	*Osmia*	*malina*
Osmia	*bella*	*Osmia*	*melanopleura*
Osmia	*brevis*	*Osmia*	*montana quadriceps*
Osmia	*californica*	*Osmia*	*nigrifrons nigrifrons*
Osmia	*cobaltina*	*Osmia*	*odontogaster*
Osmia	*coloradensis*	*Osmia*	*paradisica*
Osmia	*cyanella*	*Osmia*	*penstimonis*
Osmia	*densa*	*Osmia*	*proxima*
Osmia	*enixa*	*Osmia*	*pusilla*
Osmia	*exigua*	*Osmia*	*subaustralis*
Osmia	*indeprensa*	*Osmia*	*trevoris*
Osmia	*juxta*	*Osmia*	*tristella*
Osmia	*laeta*	*Osmia*	*visenda*

Three viruses were found in the pooled RNA samples sent to Novogene, Inc: DWV-B, CBPV, and Andrena-Associated Bee Virus-1 (AnBV-1). In analysis of the 146 wild foraging bees collected for processing, nine individuals tested positive for DWV-B and five tested positive for CBPV. This equaled an overall prevalence of 6.16% for DWV-B and 3.4% prevalence rate across all sites and time points. AnBV-1 was not able to be successfully amplified through PCR, although high read counts in the metatranscriptomics reports indicate that this was the most abundant viral transcript in our pooled samples. DWV-B was present at five sites, whereas CBPV was present at four sites. Three sites had no viruses (Table 3). For CBPV, 3 out of the 4 sites it was found at were not in close proximity to hives, whereas ⅔ of the apiary sites lacked this virus completely. The sites that lacked DWV-B were all in close proximity to Kyburz Flat meadows.

Table 3. The time points at which each virus is present at each site.

Site	CBPV Time Points	DWV-B Time Points
KFA	None	None
SHFS	3	3
PER	3	3
MMM	1 & 3	1 & 3
KFC	3	None
KFB	None	None
ORR	None	1, 3
STAMP	None	2
LAC	None	None

Virus Prevalence and Honey Bee Presence

CBPV prevalence increased by 892.54% from time point 1 to time point 3 (Figure 3A). Meanwhile, DWV-B increased by 412.04% from time point 1 to time point 3 (Figure 3B). ANOVA revealed a significant difference between time points for CBPV (F-value = 7.876, df = 1, p-value = 0.0113), but not for DWV-B (F-value = 2.801, df = 1, p-value = 0.111).

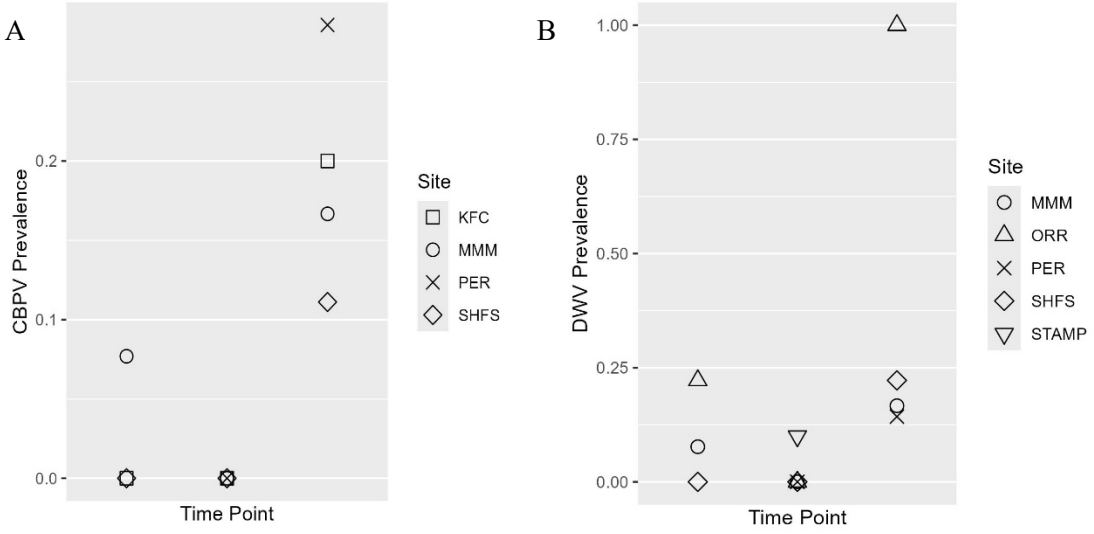

Figure 3. (A) CBPV prevalence and (B) DWV-B prevalence before honey bee arrival, shortly after arrival, and several weeks after arrival. Each graph excludes sites in which prevalence was zero at all time points.

Virus Prevalence and Honey Bee Abundance

Pearson's product-moment correlation analysis revealed that there is a statistically nonsignificant positive correlation between honey bee abundance and CBPV prevalence (r = 0.1781019, p-value = 0.5424) (Figure 4A). Similarly, there is a statistically nonsignificant positive correlation between honey bee abundance and DWV-B prevalence (r = 0.1574014, p-value = 0.591) (Figure 4B).

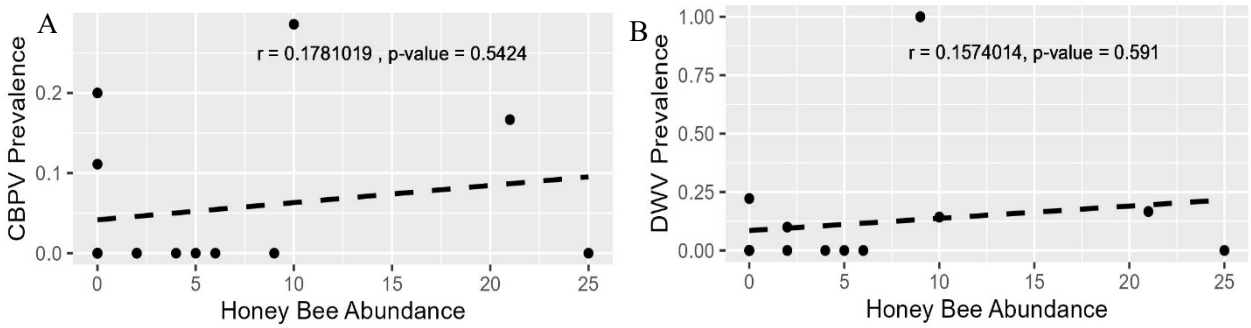

Figure 4. Pearson's correlation between honey bee abundance and (A) CBPV and (B) DWV-B prevalence.

Virus Prevalence and Apiary Proximity

At time point 2 apiary distance did not have a significant effect on DWV-B prevalence (F-value = 0.556, df = 2, p-value = 0.601) (Figure 5A). Similarly, at time point 3 apiary distance had no significant effect on either DWV-B (F-value = 0.27, df = 2, p-value = 0.78) (Figure 5B) or CBPV prevalence (F-value = 2.813, df = 2, p-value = 0.205) (Figure 5C).

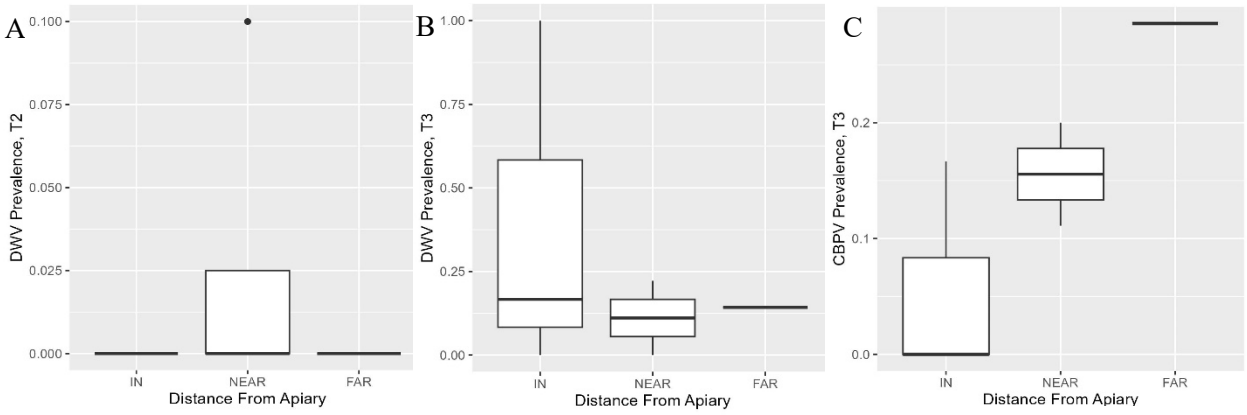

Figure 5. Box plots showing prevalence of DWV-B at time point 2 (A) and time point 3 (B), as well as CBPV at time point 3 (C) relative to the distance from nearest apiary grouped into "in, near, far".

DISCUSSION

Three viruses infected mason bees in Tahoe National Forest. DWV, CBPV, and AnBV-1 are all viruses that are commonly associated with other types of bees, particularly honey bees and mining bees (*Andrena*). Although DWV-B and CBPV have previously been found in mason bees, this study is the first to find evidence of AnBV-1 infections in mason bees. Further exploration of how these viruses are distributed throughout the study region found a significant association between higher prevalence of CBPV after honey bee arrival.

Metatranscriptomics

Metatranscriptomic sequencing revealed that there were three viruses infecting mason bees in this region of the Sierra Nevada. Interestingly, the dormant bees were found to be free of viruses suggesting a lack of vertical transmission, although this is inconclusive due to only 10 of the original 220 dormant bees being clean enough to analyze. Although it is possible that an infected adult could leave virus-contaminated pollen rations for their offspring (Mazzei et al. 2014), it is also possible that the viral RNA could degrade before the larvae hatches and consumes the contaminated food depending on various environmental factors, including humidity and temperature (Singh et al. 2010). Currently, the amount of time that these viruses remain infectious in the environment is entirely unknown. Future research may elucidate whether vertical transmission is a factor for how these viruses are spread. The relatively low number of samples placed into the pooled samples sent to Novogene Inc. could have missed viruses that are of lower prevalence, which is likely as prevalence of both honey bee associated viruses were indeed patchy amongst this focal population. Moreover, the existence of low prevalence pathogens is likely, as the numerous and diverse foraging resources dilute the exposure of bees to these viruses (Piot et al. 2021). Furthermore, DWV and other honey bee viruses have been found to be at high prevalence in non-*Osmia* wild bee populations (Jones et al. 2021). More shotgun RNA sequencing could be performed with the foraging bee samples to potentially identify more viruses. This further analysis should be accompanied by another round of cDNA analysis to understand how these new viruses are dispersed in the population.

Furthermore, this study does not identify mason bees to species, only to genus. This is largely due to the incredible similarity between different species, requiring identification from taxonomic experts. More efforts should be made to make accurate identification of mason bees easier, such as DNA barcoding. As the barcode regions of the genomes of the pinned specimens from this study become sequenced through these methods, the rest of the bees with positive viral cases could be identified to species based on their DNA barcodes and we can better understand which viruses are infecting which *Osmia* species.

Virus Prevalence and Honey Bee Presence

CBPV and DWV: Honey Bee Viruses

This study found a significant relationship between the presence of honey bees and the prevalence of CBPV. Prevalence was low before honey bee arrival, was zero immediately after arrival of the apiaries, and increased several weeks after. CBPV is another virus commonly found in honey bees that has started to be found in mason bees, specifically *O. bicornis* (Radzevičiūtė 2017). Typical symptoms of CBPV include hair loss and trembling, although it is unknown if these symptoms are experienced by bees other than honey bees, nor is it known what viral load would be necessary to exhibit these symptoms (Ribière et al. 2010). Because these bees were caught while actively foraging it is unlikely that CBPV was in high enough concentration to cause similar symptoms. Indeed, most field studies will be underestimating the prevalence of the more virulent pathogens, as we are specifically sampling bees that are healthy enough to fly and forage. However, this study does not examine whether or not there is a fitness cost to being infected by CBPV. More research is needed to understand whether viruses like CBPV have an impact on mason bee fitness.

Although DWV-B prevalence follows a similar pattern between time points to CBPV, the relationship between prevalence and time points is not significant. This is not surprising, given that DWV-B is able to infect multiple genera of bees and has been found to be the most prevalent virus in previous studies of other bee populations, including *Osmia* (Singh et al. 2010, Tiritelli et al. 2024). DWV-B is most commonly associated with honey bees, and its persistence in honey bee populations is largely due to the parasitic mite *V. destructor* (Martin et al. 2012). The mite has not

been observed in *Osmia* populations, and given the fact that the dormant bees were found free of viruses, it is likely that honey bees (or another species of bee) are spreading the pathogen to mason bees through contaminated shared floral resources (Burnham et al. 2021). The low prevalence of the virus at most sites is likely due to the ample availability of floral resources diluting transmission in the landscape (Piot et al. 2021). Future research should focus on the role of honey bees in spreading viruses to native bees, especially considering the widespread usage of commercial honey bees for agriculture which results in the movement of honey bees over vast distances.

Andrena-Associated Bee Virus-1

Despite AnBV-1 being found in the pooled sample that underwent metatranscriptomic analysis, it was not successfully detected in the individual samples. AnBV-1 was first discovered through metatranscriptomic analysis of mining bees in Israel in 2021, in which the virus was found to infect honey bees, as well (Daughenbaugh et al. 2021). My study is the first to find AnBV-1 infection mason bees. Because mason bees and mining bees are both solitary cavity nesting species, the two genera likely have many shared resources in the region that provide ample opportunity for a spillover event. Alternatively, commercial honey bee colonies from Israel may have facilitated the movement of the virus to this region. Honey bees are well known for their ability to facilitate the movement of pathogens across the globe, leading to regulations of international, and, in some cases, interstate movement of honey bees and beekeeping products (Goulson 2003, Marcelino et al. 2022). Expanding research on viruses in commercial honey bee colonies to include novel viruses such as AnBV-1 may provide insight into how this virus spread.

Interestingly, AnBV-1 was only identified in the metatranscriptomic analysis, and not during individual sequencing. Given the geographical distance between Tahoe National Forest and Israel, it is possible that the virus identified in this study is genetically distinct from the original virus. Because the primers for the amplification stage of the analysis were based on the virus from Israel, the targeted region of the primers may not have been conserved, which would explain why the virus was not identified outside of the metatranscriptomic analysis. Further analysis of this particular strain is necessary to understand why individual sequencing of the virus yielded contrasting results to the metatranscriptomic analysis. Future directions include using the sequence data we will design primers to specifically target the viral strain we have present.

Broader Implications

These findings support the speculation that honey bees are mediators of the virus to native species. However, both of these viruses were found in *Osmia* at time point 1, before honey bee arrival, which could be from a number of possibilities. (1) They could have been infected from a different species that we did not account for, (2) that this virus could have successfully overwintered in these bees and is circulating in this population without honey bees or (3) these are external contaminants and the viruses are just being picked up on the pollen they are foraging, but not starting full infections. The latter could be answered by running PCR on viral primers that specifically amplify the negative sense strand of these focal viruses. As they are all positive sense single stranded RNA viruses, the negative sense strand would only be there if the virus was replicating. Successfully amplifying the negative sense strand of any of these viruses would indicate that this virus is not just an external contaminant, but rather actively replicating in this bee. As far as the possibility of these viruses overwintering in mason bees, preliminary results we acquired from the managed *Osmia* bees showed very little evidence of any viruses, although due to the relatively small sample size pooled together for this analysis these results are inconclusive. If this pattern would hold true, and these viruses are not in overwintering in *Osmia,* then the first possibility must be true, and that these *Osmia* are picking up viruses from other unaccounted for species and proliferate as minor epidemics in foraging adults, but they are not a suitable enough primary host to allow for the virus to successfully overwinter in this species.

This study argues that the widespread distribution of honey bees has facilitated the transmission of viruses to other species and is the first to analyze the viruses of mason bees in the Sierra Nevada. Consequently, this study is the first to find another CBPV, which is a honey bee virus, and AnBV-1, a mining bee virus, in mason bees. Although the use of honey bees in agriculture is crucial for human food systems, commercial colonies are often introduced to habitats with diverse floral resources that often overlap with wild bee habitat. Although some measures are in place to restrict the movement of pathogens across landscapes, current restrictions are designed to help commercial populations and are not designed to stop the spread of pathogens to wild populations. More research is needed to understand the effects that introduced species can have on

the virology of native bees, and to minimize the impact of commercial livestock on wild populations.

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