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## Preliminary analysis shows that feral and managed honey bees in Southern California have similar levels of viral pathogens

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### ABSTRACT

Bees provide critical pollination services but are threatened by multiple stressors, including viral pathogens. The role of feral honey bees (FHB) in spreading viral pathogens is of increasing interest. We provide preliminary evidence that FHB colonies may act as persistent reservoirs of acute bee paralysis virus (ABPV), black queen cell virus (BQCV), and deformed wing virus (DWV) in southern California. Additionally, though FHB are not treated for diseases or parasites, they harbor similar pathogen loads to managed honey bees (MHB), emphasizing the need for future studies describing how FHB mitigate viral pathogen stress.

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Feral honey bees; deformed wing virus; acute bee paralysis virus; black queen cell virus; pollinator community

MHB face mounting pathogen stress (Meixner & Le Conte, 2016). To reduce infection, MHB colonies require intensive treatments, particularly to diminish the prevalence of mite-vectoring viral pathogens. However, FHB are highly successful throughout southern California without human intervention, suggesting an increased capacity to mitigate pathogen stress. Here we take a key first step to understanding the connections between honey bee-associated viruses (HBAV) in FHB and MHB in southern California by quantifying the abundance of common viral pathogens: deformed wing virus (DWV), black queen cell virus (BQCV), and acute bee paralysis virus (ABPV) among MHB and FHB throughout a year.

We collected foragers from the entrances of 3 FHB and 3 MHB colonies from San Diego and Riverside counties during September, October, November of 2019, and April, June, July, and August of 2020. MHB colonies were managed according to Honey Bee Health Coalition best practices (Honey Bee Health Coalition, 2019). We only sampled FHB by regular observation to have occupied a nesting site for at least a year by regular observation. Colonies were located at least 8 km apart to minimize forager overlap. Bees from each colony were collected into a single 15 mL centrifuge tube, euthanized on dry ice, and stored at  $-70^{\circ}\text{F}$ . To avoid contamination between sites, we used disposable gloves while collecting, and disinfected nets with 10% bleach between sites. All MHB were sampled with the permission of apiary owners. FHB colonies were all on public land.

Total RNA was extracted from whole individual bees using TRIzol<sup>TM</sup> Reagent (Life Technologies). The absolute number of viral genome equivalents (VGE) was determined via one-step qRT-PCR (iTaq<sup>TM</sup> Universal SYBR<sup>®</sup> Green One-Step; CFX Maestro platform (BioRad)), using the protocol and USR standard curve described in Carrillo-Tripp et al. (2016). Viral titers for sites and bee types were compared via repeated-measures general linear model in RStudio (R Core Team, 2019; RStudio Team, 2020), using the lme4 (Bates et al., 2015), and lmerTest (Kuznetsova et al., 2017) packages. Bee type and date of the collection were fixed effects. The site was a random effect. To improve model fit, we log-transformed VGE. Figures were produced using ggplot2 (Wickham, 2016). Data and analysis code are available via Zenodo.

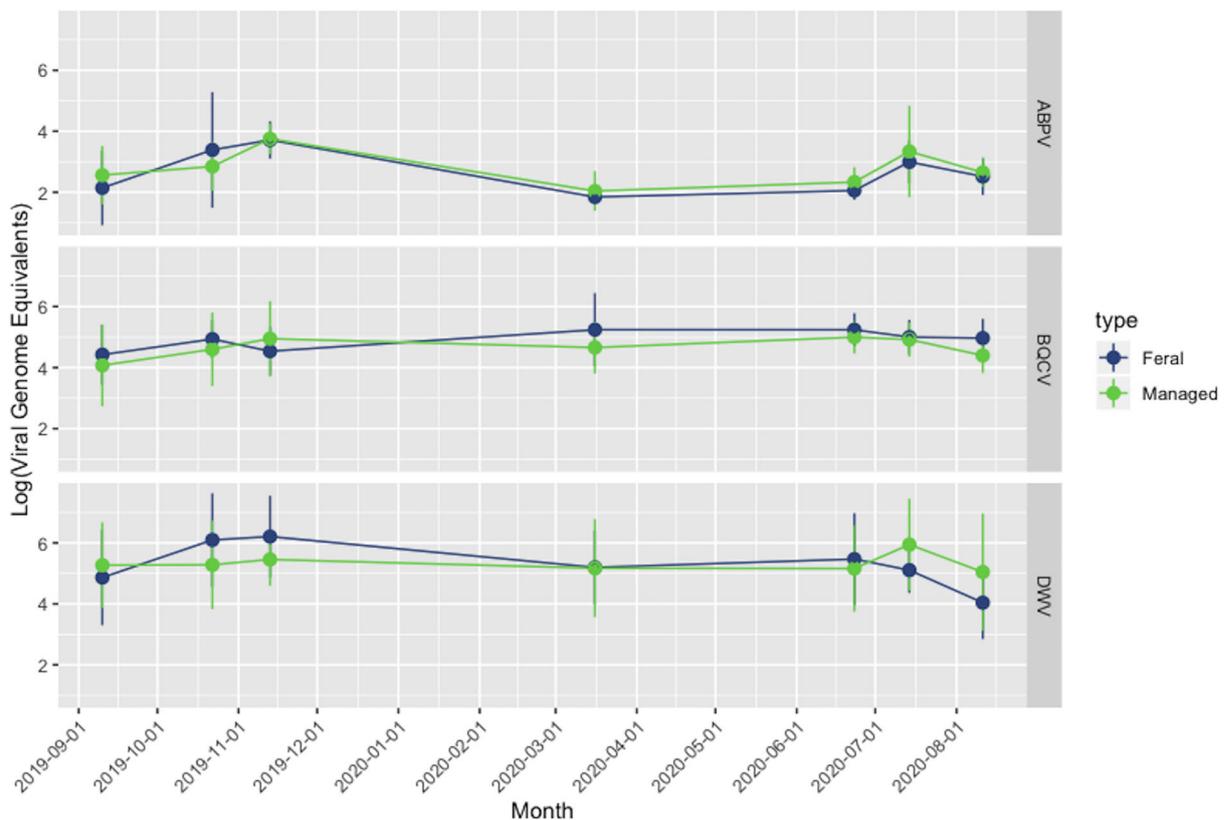
Average viral titers in our colonies fluctuated over the collection dates (Table 1), as is typical (Antúnez et al., 2015), except ABPV, which was present only in low titers, often below the level assumed to represent true infection (Dolezal et al., 2016). None of the measured viruses showed significantly different titers between FHB and MHB during any sampling period (Table 1, Figure 1).

Similar levels of HBAV in FHB and MHB in southern California suggest that both bee types are sources of HBAV for the pollinator community. Additionally, since FHB do not rely on management to mitigate pathogen stress, they may have means to combat viral stressors that MHB do not. In Pennsylvania, Hinshaw et al. (2021) also found similar, and occasionally higher, HBAV levels in FHB. The fact that FHB across North America appear to be

**Table 1.** Repeated measures regression of viral titers in FHB and MHB.

ABPV	Effect Size Estimate ( $\beta$ )	Standard Error	DF	t-value	p-value
Intercept	24.120	12.980	248.400	1.858	0.064
Bee Type	-20.230	18.250	248.400	-1.108	0.269
Date	-0.001	0.001	248.400	-1.654	0.099
Type x Date	0.001	0.001	248.300	1.114	0.267
BQCV	Estimate ( $\beta$ )	Standard Error	DF	t-value	p-value
Intercept	-22.840	10.010	248.500	-2.281	0.023
Bee Type	3.635	14.070	248.500	0.258	0.796
Date	0.002	0.001	248.100	2.773	0.006
Type x Date	0.000	0.001	248.100	-0.281	0.779
DWV	Estimate ( $\beta$ )	Standard Error	DF	t-value	p-value
Intercept	44.250	16.780	248.400	2.637	0.009
Bee Type	-44.010	23.610	248.400	-1.864	0.064
Date	-0.002	0.001	248.400	-2.326	0.021
Type x Date	0.002	0.001	248.400	1.869	0.063

Model:  $\log(\text{Viral genome equivalents of target virus}) \sim \text{Bee type} + \text{Date} + (\text{Bee type} \times \text{Date}) + (1|\text{Site})$ .



**Figure 1.** Infection intensity of feral (purple) and managed (green) honey bees. Error bars indicate standard deviations ( $n = 5$  bees per colony, except for November, where  $n = 3$  bees per colony).

reservoirs of viruses harmful to MHB and other pollinators means they likely play important roles in the pathogen dynamics among pollinator communities (McMahon et al., 2015).

In southern California, genomic admixture with *A. mellifera scutellata* (Cridland et al., 2018) in FHB populations may play a role in mitigating pathogen stress, as suggested by Tarpy (2003), and Meixner et al. (2010). Behaviors often attributed to admixture with *A.m. scutellata*, including anti-*Varroa* hygienic and grooming behaviors, increased swarming frequency, smaller colony sizes, and heightened defensiveness (Carr et al., 2020; Nganso et al., 2017; Schneider et al., 2004), may help ward

off diseases. However, neither increased genetic diversity nor African ancestry appears necessary: increased diversity within South Carolinian FHB was correlated with immune response, though FHB showed lower genetic diversity than MHB (López-Urbe et al., 2017). Behaviors associated with Africanization are found in FHB in the absence of *A.m. scutellata* ancestry (Locke, 2016; Loftus et al., 2016; Seeley et al., 2015). This suggests that feralization itself may select for beneficial adaptations to mitigate disease. Thus, FHB may be both important sources of pathogens among pollinators, and potential resources for understanding pathogen mitigation in honey bees.

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