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NOTES AND COMMENTS

Honey bee stocks exhibit high levels of intra-colony variation in viral loads

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ABSTRACT

Colonies of the western honey bee, *Apis mellifera* L., are comprised of tens of thousands of genetically related individuals that can easily share and spread pathogens. Honey bee colonies exhibit variations in disease susceptibility that can translate into highly variable pathogen replication among individuals within a colony, between colonies, and between stocks. Here, we investigate the degree of variation in viral titers of common honey bee viruses (DWV-A, DWV-B, IAPV, LSV2, BQCV) within colonies and between colonies of five honey bee stocks in the United States. Our results showed high intra-colony variation in DWV-A, DWV-B, IAPV, and LSV2, but not in BQCV titers. However, the level of variation was not consistent across stocks. Here, we empirically demonstrate that there is significant intra-colony variation in viral titers and that some stocks are more prone to this variability. Our results highlight the need for further studies comparing virus susceptibility among stocks.

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Colony variation; genetics; Deformed Wing Virus; survivor stocks

Honey bees are the most important managed pollinators for the majority of pollination-dependent agricultural crops including apples, blueberries, and almonds (Calderone, 2012). However, the annual mortality of colonies significantly impacts the profitability of beekeeping operations. These losses are often attributed to parasitic Varroa mites, *Varroa destructor* (Anderson and Trueman), and their associated viruses (van Dooremalen et al., 2012; Highfield et al., 2009). Thus, mite levels and viral titers are commonly used as biomarkers of honey bee health (López-Uribe et al., 2020). Because of the high cost and time investment in quantitative PCR, virus quantification is often based on pools of individuals from each colony, particularly for large-scale surveys from beekeepers' managed honey bee colonies (BIP https://research.beeinformed.org/). However, the degree to which intra-colony variation in viral titers among individuals can lead to biased estimates of viral titer is not well understood. Additionally, pathogens can interact with bee genotypes leading to different levels of intra-colony viral titers (Thaduri et al., 2019). Here, we take a crucial first step to providing empirical data on the field relevant level of variation in viral titers among individuals within colonies using five stocks commonly used in the United States: California, Georgia, Russian, Pennsylvania survivors, and Indiana survivors.

In June 2020, we established 15 experimental colonies (3 colonies per stock) by replacing the queens in full-size colonies with open-mated queens from one of five stocks. All queens were of the same age and purchased from commercial sources. Queens from all sources were shipped with monitors showing that the temperatures experienced during shipment were safe for queens and their stored sperm (McAfee et al., 2020). We treated all colonies with formic acid before establishing the new queens to diminish and equalize the Varroa mite populations across colonies, but no other treatments were applied in the hives. Approximately 90 days after the new queen was established, 200 adult worker bees were collected from brood frames of each colony and placed on dry ice prior to storage at −80 °C until analysis. On the day of the sampling, we conducted alcohol washes from 300 bees to account for potential differences in Varroa mites among colonies. The heads and abdomens of ten workers from each colony were individually placed in 2.0 ml tubes with eight to ten 2.0 mm BashingBeads (Zymo Research, Irvine, CA, United States), and homogenized using a BeadBlasterTM24 (Benchmark Scientific, Edison, NJ, United States) at 6.0 m/s for three 30 s intervals. RNA was extracted from the lysate with a ZYMO Quick RNA Mini-prep Kit (Zymo Research, Irvine, CA, United States) per the manufacturer's instructions and

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eluted in nuclease-free water. cDNA was synthesized from 1.5 μg of RNA, random primers, and MultiScribe RT, according to the manufacturer's protocol (Applied Biosystems, Foster City, CA, United States).

We quantified viral titers from individual workers *via* qRT-PCR of the five viruses with the highest prevalence for the state of Pennsylvania (USA) (BIP https://research.beeinformed.org/): Deformed Wing Virus A (DWV-A), Deformed Wing Virus B (DWV-B), Israeli Acute Paralysis Virus (IAPV), Black Queen Cell Virus (BQCV), and Lake Sinai Virus 2 (LSV2) (Table S1). EF1-α was used as a reference gene and as a cDNA quality control. qPCR was performed in 384-well plates using a QuantStudio 5 Real-Time PCR System (Applied Biosystems). Each reaction contained 2 μl of cDNA or negative template, 0.25 μl of each of the forward and reverse primers (10 μM), 2.5 μl of nuclease-free water, and 5 μl of PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, Foster City, CA, United States) as per Hinshaw et al. (2021). Target genes were run in triplicates, and standard curve samples were run in duplicates due to plate size limitations. Relative quantification for each gene was calculated through the $2^{-\Delta\Delta CT}$ method using EF1-α and the samples with the highest Cq value as references (Livak & Schmittgen, 2001). We used linear mixed models to estimate the effect of stock on the mean and variance of viral titers. Specifically, we fit a mixed-effects model with stock as a fixed effect, colony as a random intercept, and distinct residual variance parameters across stocks using the *lme* function in the R package *'nlme'* in R version 4.2.0. We used a likelihood ratio test to compare models with distinct residual variance against a reduced model with homogeneous variance across stocks. We corrected *p*-values across all pairwise comparisons using the sequential method of Holm (1979), to control the family-wise error rate.

Overall, virus detection was high for DWV-A (140/150 workers), DWV-B (127/150), and BQCV (143/150) and low for IAPV (27/150) and LSV2 (29/150). Models with heterogeneous variance across stocks better explained the variation in viral titers for DWV-A (LRT = 22.93, *p*-value < 0.001), DWV-B (LRT = 30.69, *p*-value < 0.001), IAPV (LRT = 52.85, *p*-value < 0.001), LSV2 (LRT = 121.32, *p*-value < 0.001), but not BQCV (LRT = 3.04, *p*-value = 0.552) (Table 1). Workers from colonies established from Georgia queens showed higher variance for DWV-A, while workers from colonies with California queens showed higher variance for DWV-A and DWV-B. Workers from colonies with Russian and Indiana queens showed higher variance for LSV2 (Figure 1; Table S2). Stock significantly affected mean viral titers for DWV-A ($F_{4,10}$ = 3.99, *p*-value = 0.0347), but not for the other viruses (Table 1). The average number of mites per colony was equal among stocks ($F_{4,10}$ = 0.566, *p*-value = 0.693; Table S3).

Table 1. Summary of the results of the mixed linear models estimating intra-colony variation and effect of stock on viral titers.

	Effect on variance of titer		Effect of average titer	
Virus	$LRT_{7,11}$	*p*-value	$F_{4,10}$	*p*-value
DWV-A	22.93	< 0.001	3.99	0.0347
DWV-B	30.69	< 0.001	2.20	0.143
BQCV	3.04	0.552	1.25	0.352
IAPV	52.85	< 0.001	1.669	0.233
LSV2	121.32	< 0.001	3.14	0.0644

Intra-colony variation was estimated through a likelihood ratio test (LRT) comparing models with fixed and heterogeneous variances. The effect of stock was estimated through an ANOVA of mixed linear model with stock as a fixed effect and colony as a random effect.

Our study demonstrates that viral titers are generally highly variable among colony individuals, but this variation is more pronounced in some stocks. Because all colonies were located in the same apiary with a similar potential for between-colony transmission, our results suggest that either different stocks perform differently when exposed to these viruses (e.g., through horizontal transmission *via* an oral-fecal route or Varroa mites) or that queens of some stocks arrived at the apiaries with a higher prevalence of viruses that were later transmitted to the rest of the colony (vertical transmission). Variation in viral titers within colonies was significant for all viruses except for BQCV, which appears to be an endemic virus in honey bee colonies in Pennsylvania as it was detected in 95% of individuals across all colonies and at similar levels.

Stock was only an important predictor of viral titers for DWV-A and marginally significant for LSV2. Our results showed that, among the five stocks compared, Georgia and California stocks show the highest viral titers for DWV-A. The DWV complex (DWV-A and DWV-B) is transmitted by the ectoparasitic mite *V. destructor* (Posada-Florez et al., 2019). Therefore, the low observed levels of DWV titers in the Russians, Pennsylvania survivors, and Indiana survivors are likely the result of breeding for Varroa mite resistance in these stocks (Morfin et al., 2020; Rinderer & Guzman, 2001). However, the direct connection between differences in virus and mite levels among stocks should be explicitly investigated in the future. The detection of IAPV and LSV2 was low across all colonies and stocks, making it difficult to draw strong conclusions about differential stock susceptibility to these viruses.

Even with evidence demonstrating that honey bee stocks vary in their response to biotic and abiotic factors (Khongphinitbunjong et al., 2015; Milone et al., 2020), most honey bee studies do not account for genetic stocks in their experimental design. This omission leaves a gap in knowledge on the magnitude of the effect of genetics on how honey bees respond to environmental stressors and pathogens. With more admixture of stocks through breeding

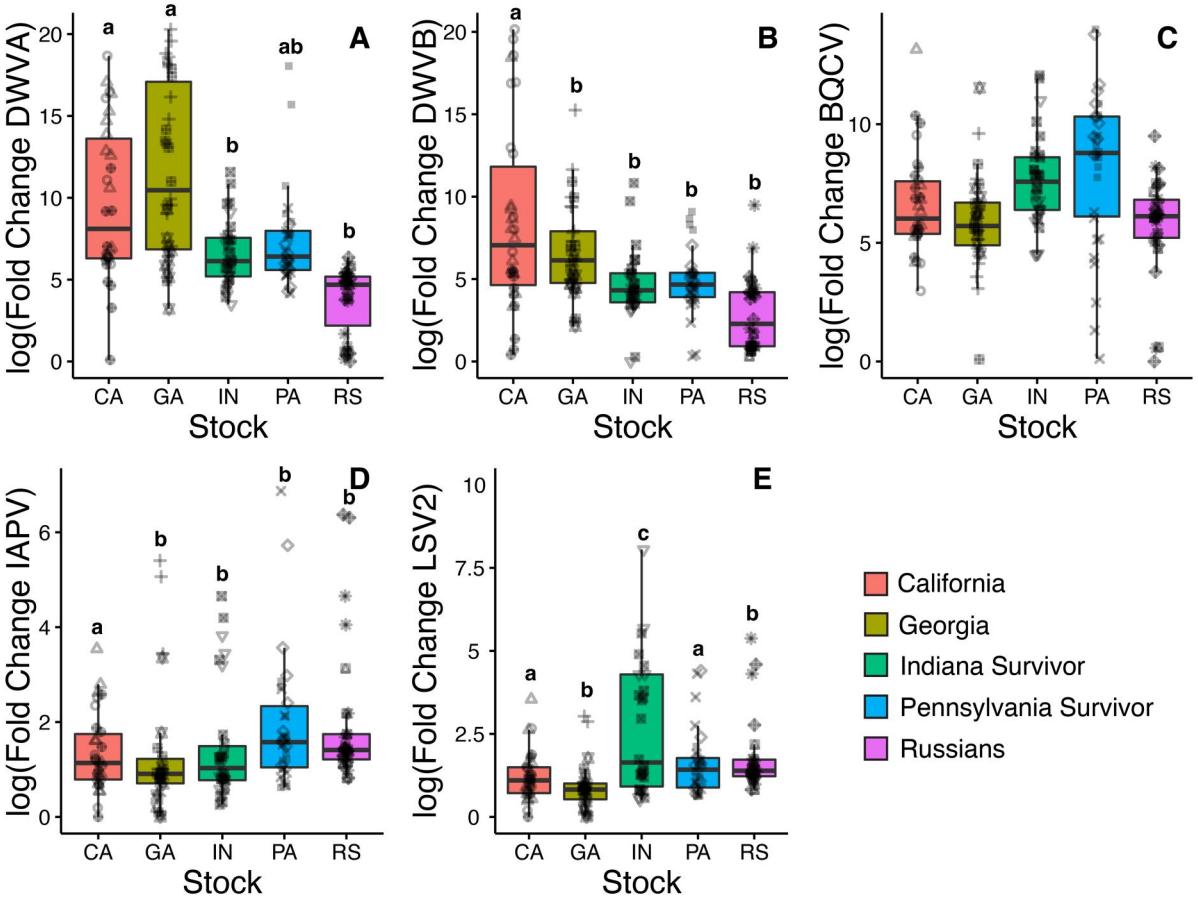

Figure 1. Boxplots showing the relative abundance of five viruses DWV-A, DWV-B, BQCV, IAPV, and LSV2 in workers from five stocks. Each boxplot includes viral titers for 10 individuals from 3 colonies per stock ($n = 30$). Different symbols are used to represent individuals from different colonies. Relative abundance was estimated as the log of the $2^{-\Delta\Delta Ct}$ average values from three technical replicates. Letters indicate significant differences between stocks. Outliers are not shown for IAPV and LSV2 to improve the readability of the figure.

and natural swarming, it is important to continue to compare phenotypic traits among stocks over multiple generations. Our results highlight the need to understand stock performance, especially for desirable traits, such as mite and pathogen resistance, to help beekeepers make informed decisions about choices for purchasing queens from different sources. In addition, our findings demonstrate that pooling samples can be misleading when estimating colony levels of viral infections in some cases.

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Disclosure statement

The authors declare no potential competing interests.

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Data availability statement

Raw data and code for statistical analyses are included in the supplementary data files.

Suppl-Cambron-etal-2024-TJAR_DataAnalysis.R
Suppl-Cambron-etal-2024-TJAR_rawdata.csv

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