Homesick: Impacts of migratory beekeeping on honey bee (*Apis mellifera*) pests, pathogens, and colony size

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Honey bees are important pollinators of agricultural crops and the dramatic losses of honey bee colonies have risen to a level of international concern. Potential contributors to such losses include pesticide exposure, lack of floral resources and parasites and pathogens. The damaging effects of all of these may be exacerbated by apicultural practices. To meet the pollination demand of US crops, bees are transported to areas of high pollination demand throughout the year. Compared to stationary colonies, risk of parasitism and infectious disease may be greater for migratory bees than those that remain in a single location, although this has not been experimentally established. Here, we conducted a manipulative experiment to test whether viral pathogen and parasite loads increase as a result of colonies being transported for pollination of a major US crop, California almonds. We also tested if they subsequently transmit those diseases to stationary colonies upon return to their home apiaries. Colonies started with equivalent numbers of bees, however migratory colonies returned with fewer bees compared to stationary colonies and this difference remained one month later. Migratory colonies returned with higher black queen cell virus loads than stationary colonies, but loads were similar between groups one month later. Colonies exposed to migratory bees experienced a greater increase of deformed wing virus prevalence and load compared to the isolated group. The three groups had similar infestations of *Varroa* mites upon return of the migratory colonies. However, one month later, mite loads in migratory colonies were significantly lower compared to the other groups, possibly because of lower number of host bees. Our study demonstrates that migratory pollination practices has varying health effects for honey bee colonies. Further research is necessary to clarify how migratory pollination practices influence the disease dynamics of honey bee diseases we describe here.

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22 **Abstract**

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24   colonies have risen to a level of international concern. Potential contributors to such losses

25   include pesticide exposure, lack of floral resources and parasites and pathogens. The damaging

26   effects of all of these may be exacerbated by apicultural practices. To meet the pollination

27   demand of US crops, bees are transported to areas of high pollination demand throughout the

28   year. Compared to stationary colonies, risk of parasitism and infectious disease may be greater

29   for migratory bees than those that remain in a single location, although this has not been

30   experimentally established. Here, we conducted a manipulative experiment to test whether viral

31   pathogen and parasite loads increase as a result of colonies being transported for pollination of a

32   major US crop, California almonds. We also tested if they subsequently transmit those diseases

33   to stationary colonies upon return to their home apiaries. Colonies started with equivalent

34   numbers of bees, however migratory colonies returned with fewer bees compared to stationary

35   colonies and this difference remained one month later. Migratory colonies returned with higher

36   black queen cell virus loads than stationary colonies, but loads were similar between groups one

37   month later. Colonies exposed to migratory bees experienced a greater increase of deformed

38   wing virus prevalence and load compared to the isolated group. The three groups had similar

39   infestations of *Varroa* mites upon return of the migratory colonies. However, one month later,

40   mite loads in migratory colonies were significantly lower compared to the other groups, possibly

41   because of lower number of host bees. Our study demonstrates that migratory pollination

42   practices has varying health effects for honey bee colonies. Further research is necessary to

43 clarify how migratory pollination practices influence the disease dynamics of honey bee diseases

44 we describe here.

Introduction

46   Animal-mediated pollination, provided primarily by bees, is required for the production

47 of 75% of agricultural food crops (Klein et al., 2007) and provides an estimated annual value of

48 $200 billion worldwide (Gallai et al., 2009). Managed honey bees (*Apis mellifera*) are the most

49 important commercially available pollinator and contribute approximately $17 billion in

50 pollination services revenue annually to the United States (US) alone (Calderone, 2012).

51 However, for over a decade, honey bees have experienced elevated colony losses (Neumann &

52 Carreck, 2010; Potts et al., 2010; van der Zee et al., 2012, 2013; Kulhanek et al., 2017) attributed

53 to multiple threats including pesticide exposure (Tsvetkov et al., 2017; Woodcock et al., 2017),

54 forage availability (Decourtye, Mader & Desneux, 2010), and numerous pests and pathogens

55 (vanEngelsdorp & Meixner, 2010). The numerous threats affecting honeybees may be

56 exacerbated by practices inherent to the apicultural industry and required for large-scale crop

57 pollination, specifically migratory beekeeping (Royce & Rossignol, 1990; Traynor et al., 2016a).

58   To meet the pollination demand of a variety of US agricultural crops, large numbers of

59 bees are moved among crops at regional and national scales. Conditions for migratory colonies

60 vary greatly depending on the distance traveled and the crops visited. In the most extreme cases,

61 colonies are transported by truck to a series of monoculture crops including blueberries,

62 cranberries, almonds, and citrus (VanEngelsdorp et al., 2013) for months at a time. At each stop

63 along the journey, millions of bees from different origins converge on a single crop for the

64 duration of bloom, which typically lasts approximately one month and may offer little forage

65   diversity (Decourtye, Mader & Desneux, 2010; Colwell et al., 2017). Nectar, comprised of

66   sugars and amino acids, is required to fuel flight and feed the colony while pollen, high in

67   protein and fats, provisions developing brood (Brodschneider & Crailsheim, 2010a). To ensure

68   survival en route or when crops are not in bloom, colonies may be supplemented with sucrose

69   syrup and artificial pollen, temporary but poor substitutes for the diverse array of nectar and

70   pollen types bees obtain in natural landscapes (Huang, 2012). Thus, compared to their stationary

71   counterparts, migratory colonies experience greater stress (Simone-Finstrom et al., 2016), greater

72   exposure to pesticides (Mullin et al., 2010; Traynor et al., 2016a), and lower quality forage

73   (Brodschneider & Crailsheim, 2010b; Decourtye, Mader & Desneux, 2010; Colwell et al., 2017),

74   all of which may increase susceptibility to disease (Di Pasquale et al., 2013; Sánchez-Bayo et al.,

75   2016). It is well known that stress from long distance travel results in heightened bacterial and

76   viral infections in vertebrate livestock (Yates, 1982). However, despite the importance of large-

77   scale pollination events for agriculture, few studies have examined how migratory conditions

78   may contribute to disease incidence in bees (Welch et al., 2009; Zhu, Zhou & Huang, 2014;

79   Traynor et al., 2016b).

80   In the US, there are an estimated 2.62 million commercial honey bee colonies of which

81   over half are contracted for crop pollination (USDA National Agricultural Statistics Service,

82   2017b). California almond pollination is the largest annual event for the migratory beekeeping

83   industry, requiring over 1.5 million honey bee colonies (USDA National Agricultural Statistics

84   Service, 2017a). It is the largest convergence of honey bee colonies in the US, providing

85   conditions in which pathogens are likely to be introduced, transmitted, and subsequently spread

86   as colonies move along their human-imposed migration route (Bakonyi et al., 2002; Welch et al.,

87   2009; Runckel et al., 2011; Goulson et al., 2015). Each acre of almonds requires an average of

88 two honey bee colonies (Carman, 2011) and as bees will forage 3 km from their colonies

89 (Visscher & Seeley, 1982; Beekman & Ratnieks, 2000; Couvillon et al., 2015), bees in large

90 orchards could theoretically share flowers with bees from nearly 56,000 other colonies. While

91 almond flowers may produce a large quantity of nectar and pollen, there is evidence that it is

92 relatively low quality (and possibly toxic) forage for honey bees (London-Shafir, Shafir &

93 Eisikowitch, 2003; Kevan & Ebert, 2005); moreover, the vast fields provide little forage

94 diversity for bees and are heavily sprayed with pesticides (California Department of Pesticide

95 Regulation, 2017), exposing bees to additional stress.

96   The spread of the most devastating honey bee parasites and pathogens has mainly

97 occurred as a result of transporting honey bees long distances. For example, the *Varroa* mite

98 (*Varroa destructor*), an ectoparasite and known vector of numerous RNA viruses, became a

99 major contributor to colony losses in both North America and Europe after its introduction from

100 Asia (Rosenkranz, Aumeier & Ziegelmann, 2010; Nazzi et al., 2012). *Nosema ceranae*, a

101 microsporidian implicated in high colony mortality in Spain (Higes et al., 2008), has also

102 reached high frequencies since its introduction from Asia to the Americas and Europe (Klee et

103 al., 2007; Chen et al., 2008). Despite the role of long-distance travel in disease spread, there is a

104 surprising lack of studies examining the role of migratory beekeeping in disease spread.

105   A limited number of observational surveys have compared disease loads of colonies

106 belonging to migratory and stationary operations and found a higher prevalence of some

107 pathogens in migratory colonies (Traynor et al., 2016b) including *Nosema ceranae* (Zhu, Zhou &

108 Huang, 2014) and RNA viruses (Welch et al., 2009), some of which were not previously

109 described in honey bees (Runckel et al., 2011). However, the focus of previous studies has been

110 the collection of baseline disease data to characterize diseases in migratory colonies and, as such,

111   rarely control for migratory conditions, management practices, and sampling times, all of which

112   can significantly affect disease loads and colony health (Runckel et al., 2011; Glenny et al.,

113   2017). Furthermore, studies examining the impact of migratory conditions on bees rarely include

114   a control group of stationary colonies for comparison (but see Zhu, Zhou & Huang, 2014;

115   Simone-Finstrom et al., 2016). Although migratory honey bee colonies are implicated as disease

116   sources and could serve to introduce disease to local stationary honey bee colonies (Welch et al.,

117   2009) we are unaware of previous studies that explicitly test the role of migratory colonies in the

118   spread of diseases or parasites. Here, we conducted a two-pronged experiment in which we

119   controlled for migratory conditions, sampling time, and beekeeper management practices. We

120   first tested the effects of migration on honey bee colony population size, *Varroa* mite parasites,

121   and pathogens including *Nosema* (a microsporidian) and three RNA viruses: black queen cell

122   virus (BQCV), deformed wing virus (DWV), and Israeli acute paralysis virus (IAPV). We

123   examined differences in the parasite and pathogen prevalence and load as well as colony size of

124   migratory and stationary colonies. Second, we examined if there is evidence for the transmission

125   of diseases from migratory colonies to stationary colonies. If migration exposes bees to stressors

126   that increase disease susceptibility, we predicted that migratory colonies would have greater

127   pathogen prevalence and loads when compared to their stationary counterparts, and that

128   pathogen loads in sympatric stationary colonies would increase after foraging alongside the

129   migratory colonies for one month.

Materials and Methods

131   In February 2017, we selected 48 colonies from a North Carolina apiary that is used for

132   the production of products (honey, colonies, etc.) rather than pollination services, and assigned

133   each to one of the following groups: *migratory* (n=16), *isolated stationary* (*isolated*) (n=16), and

134   *exposed stationary* (*exposed*) (n=16; Fig. 1). We transported colonies in the migratory group

135   from Whiteville (Columbus County), North Carolina to Coalinga (Fresno County), California

136   (36°21'N, 120°12'W) to pollinate almonds for the duration of the bloom (approximately one

137   month). They were then transported back to North Carolina. As typical of migratory beekeeping

138   practices, the migratory colonies were covered by netting during transport (to reduce escapees)

139   and temporarily brought to a nearby holding yard in California before and after pollinating

140   almond orchards. The isolated stationary group remained in North Carolina (34°22'N, 78°36'W)

141   and outside the flight distance from returning migratory colonies for the entirety of the

142   experiment. To maintain similar colony densities at the isolated stationary and migratory yards,

143   there were an additional 15 stationary colonies in the isolated yard. These colonies originated

144   from the same North Carolina apiary and were not tested as part of the experiment.

145   At the start of the experiment in February 2017, all colonies had 7-9 frames of bees, and

146   7-8 frames with brood. To measure bee population size, we counted frames of adult bees (FOB)

147   by assessing the coverage of adult bees on each frame and summing the estimates for all frames

148   in the brood chamber (the lower hive body containing the queen and brood) (DeGrandi-Hoffman

149   et al., 2016). Frames with brood were assessed by counting the total number of frames containing

150   30% capped brood. Each colony was provided a new queen by replacement with open-mated

151   Italian (*A. mellifera ligustica*)/ Carniolan (*A. mellifera carnica*) queens in summer 2016.

152   Colonies were matched in triplicate by frames of bees and frames of brood and randomly

153   assigned a treatment group (migratory, isolated stationary, or exposed stationary) to ensure equal

154   distribution across groups. Prior to the start of the experiment, in October 2016, we treated all

155   colonies for *Varroa* mites with fluvalinate, a synthetic pyrethroid commonly used as an acaricide

156   in honey bee colonies. No other mite or pathogen treatments were used for the duration of the

157 experiment. To ensure that colonies would persist for the duration of the experiment, we

158 provided supplemental feed to all colonies (in all treatment groups) on two occasions: pollen

159 substitute prior to shipping the migratory colonies to California and upon return, 5 lbs. of fondant

160 (sucrose and water stabilized with gelatin). As colonies grew during the duration of the study,

161 additional hive bodies were added as needed to prevent swarming.

162   We compared bee population size and disease loads in the migratory and isolated

163 stationary group three times: before the migratory group departed for California (Jan. 25),

164 immediately after the migratory group returned to North Carolina from California (Feb. 28), and

165 one month later (March 25). To test for disease spread from the migratory colonies to their

166 stationary counterparts, we monitored the third group of colonies, the exposed stationary group,

167 which remained in North Carolina but shared a yard with the migratory colonies once they

168 returned from California (34°11'N, 78°46'W). We assessed bee population size and disease loads

169 in the exposed stationary group twice: once before sharing a yard with the migratory group (Feb.

170 28), and again approximately one month after residing with the migratory colonies in the same

171 yard (March 25). Land cover surrounding each of the North Carolina yarding areas were

172 dominated by crops, mixed forest, and woody wetlands, and we expect that colonies in the two

173 sites had similar access to early spring floral resources. Hives were housed on private land and

174 permission was granted by the owners.

175   At each sampling event, we inspected all colonies for brood diseases, measured colony

176 size, and collected bees for pathogen analyses. To estimate colony size, we measured frames of

177 bees (FOB) as before (DeGrandi-Hoffman et al., 2016). We also recorded the queen status of

178 each colony (queen-right, queenless, queen cells present, or drone-laying queen). We collected

179 live bees from the brood chamber to detect and quantify the following parasites and pathogens:

180   *Varroa*, *Nosema,* BQCV, DWV, and IAPV. To quantify *Varroa* and *Nosema* spp., we collected

181   approximately 300 bees from the brood chamber and transferred them to ethanol. To quantify

182   virus prevalence and load, we collected an additional 150 bees from the brood chamber. These

183   samples were stored and shipped to Vermont on dry ice and transferred to -80°C for storage prior

184   to analysis.

185   To examine differences in climate and weather conditions experienced by the migratory

186   and stationary groups, we used publicly available NOAA local climatology data collected by

187   weather stations nearest to our field sites (NOAA National Centers for Environmental

188   Information) (Table S1).

189   ### *Varroa* mite and *Nosema* spp. quantification

190   To calculate the number of *Varroa* mites per 100 bees, ethanol samples were agitated for

191   60 seconds, strained through hardware cloth to separate the mites from the bees, and all mites

192   and bees were counted (Lee et al., 2010). We conducted spore counts to quantify *Nosema* spp.

193   Although our methods did not differentiate between the two species of *Nosema, (N. apis and N.*

194   *ceranae*) previous work has found *N. ceranae* to be the predominant species in many regions

195   (Klee et al., 2007; Chen et al., 2008; Williams et al., 2008, 2014). To conduct spore counts, we

196   transferred 100 bees from the ethanol sample to a plastic bag and pulverized them using a pestle

197   on the outside of the bag for 90 seconds. We then added 100 mL of distilled water, allowed it to

198   settle for 45 seconds, and transferred 10 µL onto a haemocytometer counting chamber. We

199   counted spores for each sample twice under 40X magnification, averaged them, and converted to

200   spores/bee (Fries et al., 2013).

201   ### Virus quantification

202   To quantify BQCV, DWV and IAPV, we transferred 50 honey bees/sample on liquid

203   nitrogen and homogenized them in an extraction bag with 10 mL of GITC buffer using protocols

204   established by USDA-ARS Bee Research Lab Beltsville, MD (Evans, 2006). We followed

205   EZNA Plant RNA Standard Protocols (Omega Bio-Tek) with 100 µL of the resulting

206   homogenate thereafter. Using a Spectrometer (Nanodrop, Thermo Scientific), we assessed all

207   RNA quantity and quality and diluted all RNA extractions to 20 ng/µL prior to virus assays.

208   For reverse transcription of RNA and absolute quantification, we performed duplicate

209   reverse transcription quantitative polymerase chain reaction (RT-qPCR) for each sample with a

210   SYBR green one-step RT-qPCR kit in 10 µL reactions using the following thermal cycling

211   program: 10 min at 50°C (RT) followed by 1 min at 95°C, and 40 amplification cycles of 95°C

212   for 15 s, 60°C for 60s. Lastly, we obtained the melt-curve starting at 65-95°C (0.5°C increments,

213   each 2 seconds). We used primers specific to the positive strand of the following RNA virus

214   targets: BQCV, DWV and IAPV, and a housekeeping gene (Actin) as a positive control of RNA

215   extraction efficiency (Table S2). We calculated quantification using duplicate standard curves of

216   gBlocks Gene Fragments (Integrated DNA Technologies; Data S1) that were developed using

217   double-stranded, sequence verified genomic blocks consisting of the four targets of interest

218   separated by ten random base pairs. Sequences of random base pairs consisting of at least 50% G

219   and Cs were used at the beginning and terminal ends of the fragment. Efficiencies were 95.21%

220   (BQCV), 91.06% (DWV), 90.27% (IAPV), and 90.12% (Actin), with correlation coefficients

221   (R^2) ranging from 0.993-0.999. To verify RT-PCR analyses, sequences with lengths of 100-130

222   bps were generated through DNA sequencing performed in the Vermont Integrative Genomics

223   Resource using a 3130xl Genetic Analyzer.

224   **Data reporting**

225   We use "pathogen prevalence" to refer to the percentage of colonies positive for a

226   pathogen (*Varroa, Nosema,* BQCV and DWV). In addition to presence/absence data, we

227   investigated the severity of infection by quantifying each pathogen—we refer to this as

228   "pathogen load". Virus load (BQCV and DWV) results for each colony are presented in average

229   virus genome copies/bee. We did not detect IAPV in our experimental colonies and it was

230   therefore excluded from further analysis. We report *Varroa* as the number of mites per 100 bees

231   and *Nosema* as average number of spores/bee.

232   .

233   **Data analysis and statistics**

234   Before analyzing, we checked all response variables for normality using Shapiro-Wilk

235   tests. To improve normality, *Varroa* and *Nosema* loads as well as BQCV and DWV loads

236   (genome copies per bee) were log + 1 transformed. To establish that there were no differences

237   between treatment groups at the outset, we analyzed all variables at the initial time step using

238   ANOVAs for continuous variables (FOB, load of *Varroa, Nosema,* BQCV, and DWV) and Chi-

239   Square tests of independence for binary variables (prevalence of *Varroa, Nosema,* BQCV, and

240   DWV*)*.

241   To test whether the full suite of response variables collectively predicted colony

242   treatment membership, we conducted classification analyses for Experiments 1 (*migratory* vs.

243   *stationary*) and 2 (*exposed* vs. *isolated*) using linear combinations based on all response variables

244   (except BQCV prevalence as it was fixed at 100% prevalence for all groups and as such caused

245   model fitting failures). To examine how groups differed after experimental manipulation, we

246   used data from sampling events two and three for Experiments 1 and 2, respectively. The models

247   were trained using a conservative cross validation approach to reduce over-fitting the model to

248 our data. We tested for differences between groups' centroids in multivariate space for each time

249 point with PERMANOVA, a non-parametric MANOVA, using Euclidian distance-based

250 dissimilarity matrices. To visualize between-group separation, the centered values from linear

251 discriminate functions (LD1 and LD2) were plotted for each colony.

252   To test the effect of treatment and time on prevalence, we analyzed all pathogens

253 (*Nosema, Varroa,* BQCV, and DWV) using separate generalized linear mixed effects models

254 (GLMMs) using the binomial (link="logit") distribution family. For measures of pathogen load,

255 and FOB, we used linear mixed effects models (Harrison et al., 2018). All models used the same

256 repeated measures design. Treatment, sampling event, and their interaction were included as

257 fixed effects in order to determine how each dependent variable was affected by our

258 manipulation through time. Colony and bee yard were included as random effects in order to

259 determine the among colony variance within each treatment and account for potential differences

260 between bee yards. To examine how the *Varroa* load of migratory and stationary colonies

261 differed over time with respect to FOB, we conducted a separate linear mixed effects model. We

262 first tested for temporal autocorrelation in the residuals of the model using an ACF plot and no

263 autocorrelation was detected. For this model, we used FOB, treatment, time, and the resultant

264 interactions as fixed effects and colony as a random effect. Significance for all models was

265 determined using Type II Wald Chi-Square tests.

266   To examine potential differences in climate between California and North Carolina

267 during the 27 days the migratory bees were in California, we used one-way Analysis of Variance

268 (ANOVAs) on average daily temperature, precipitation, and wind speed by state (NOAA

269 National Centers for Environmental Information).

270   We conducted all statistical analyses using the statistical software "R" (R version 3.3.1).

271   GLMMs were conducted using the lme4 package (v 1.1-13) (Bates et al., 2015). The

272   corresponding Type II Wald Chi-Square tests were conducted using the Anova function in the

273   car package (v 2.1-4) (Fox & Weisberg, 2011). Temporal autocorrelation was tested using the

274   acf function. Classification analyses were conducted using the lda function in the mass package

275   (v 7.3-45) (Ripley & Venables, 2002). The adonis function was used to perform PERMANOVA

276   in the vegan package (v 2.4-3) (Oksanen et al., 2017).

Results

278   While in California, migratory colonies experienced similar weather conditions (mean

279   daytime temperature, wind speed, and precipitation) to those experienced by stationary colonies

280   in North Carolina ($F_{1,52} < 3.106$, $P > 0.084$; Table S1). All colonies were absent of IAPV. BQCV

281   was present in all colonies for the duration of the study (Fig. S1).

Experiment 1: Migratory verses Stationary

283   At the start of the experiment, there was no significant difference between migratory and

284   stationary colonies in prevalence ($\chi_1^2 < 1.143$, $P > 0.285$) or load ($F_{1,30} < 3.01$, $P > 0.093$) of any

285   of the four pathogens. In addition, there was no difference in FOB at the beginning of the

286   experiment (migratory: 7.94 ± 0.57 sd, stationary: 7.44 ± 0.51 sd).

287   Upon the return of the migratory colonies, our pathogen and hive population

288   measurements collectively predicted whether a colony was migratory or stationary (Fig. 2A).

289   The linear combination (LD1) adequately discriminated between the migratory group and the

290   stationary group and yielded correct classification rates of 87.5% for migratory colonies and 75%

291   for stationary colonies. Also, prior to contact with the migratory colonies, the exposed colonies

292   were similar to the isolated stationary colonies and essentially formed one large group (Fig 2A).

293   After contact with migratory colonies, there was statistically significant group separation

294   between migratory and stationary treatments ($F_{1,30} = 5.03$, P = 0.007).

295   Migratory colonies returned from California with significantly higher BQCV loads

296   compared to the stationary group ($\chi_1^2 = 16.488$, P < 0.001; Fig 3A), and BQCV load increased

297   with time (Fig. 3A and Table 1). The prevalence (Fig. S1) and load of DWV (Fig 3B) did not

298   differ between treatments following return of migratory colonies but both increased with time

299   (Table 1). *Nosema* load and prevalence (Fig. S1) did not differ between treatments following

300   return of migratory colonies and *Nosema* load decreased with time (Table 1). However, for

301   *Varroa*, there was a significant treatment × time interaction (Fig 3C). *Varroa* loads increased

302   steadily for stationary colonies, but decreased in migratory colonies over the month after

303   returning from California ($\chi_1^2 = 6.465$, P = 0.011). There was also a significant interaction of

304   treatment × time for FOB, with migratory colonies returning with fewer FOB than their

305   stationary counterparts ($\chi_1^2 = 5.651$, P = 0.017). There was a significant interaction of FOB ×

306   treatment × time on *Varroa* loads ($\chi_1^2 = 4.045$, P = 0.044) indicating that *Varroa* loads were

307   differentially affected by FOB for each treatment group over time. Other interaction terms were

308   not statistically significant (Table 1).

309   **Experiment 2: Exposed verses Isolated**

310   At sampling event two, there was no significant difference between exposed and isolated

311   stationary colonies in pathogen prevalence ($\chi_1^2 < 1.143$, P < 0.285) or load ($F_{1,30} < 1.279$, P >

312   0.267). FOB was similar between groups at the beginning of the experiment ($F_{1,29} = 0.858$, P =

313   0.362).

314   One month after the exposed group foraged alongside the migratory colonies, there was

315   an increase in between-group separation with groups becoming more distinguishable from each

316   other. While all groups separated in this third time step, the exposed and migratory groups were

317   less distinguishable from one another compared to the stationary group (Fig. 2B). The linear

318   combinations (LD1 and LD2) yielded a correct classification rate of 75% for stationary colonies

319   but correct classification rates for migratory and exposed colonies were lower, 43.75% and

320   56.25%, respectively. PERMANOVA results indicated statistically significant group separation

321   between isolated, migratory and exposed treatments ($F_{2,43} = 4.72$, P = 0.001).

322   We found no effects of treatment (exposed verses isolated) for any of the parasite or

323   disease response variables (Fig 3). However, *Varroa* prevalence and load, *Nosema* prevalence

324   and load, and BQCV significantly increased with time (Table 2). There was a significant

325   treatment × time interaction for both DWV load ($\chi_1^2 = 9.229$, P = 0.002; Fig 3B) and DWV

326   prevalence ($\chi_1^2 = 4.94$, P = 0.026; Fig. S1) such that DWV in exposed colonies increased at

327   significantly higher rates than the isolated group. There was also a significant treatment × time

328   interaction for FOB ($\chi_1^2 = 9.946$, P = 0.0016; Fig 3D) with exposed bees increasing at a

329   significantly higher rate compared to the isolated group. Other interaction terms were not

330   significant (Table 2).

331   Discussion

332   Migratory pollination services are an essential component of the US agricultural

333   economy, yet this practice exposes honey bee colonies to a combination of factors that may

334   compromise individual bee and colony health. Although there is widespread concern that

335   migratory pollination can place honey bee colonies at increased risk to acquire and spread

336   pathogens and parasites, there is a lack of experimental evidence demonstrating this

337   phenomenon. Here, we controlled for management practices and starting conditions as well as

338   the time at which bees were sampled for diseases and parasites. Our results show that while

339   migratory conditions can negatively affect colony health and increase disease load, in some cases

340   these impacts were transient.

341   With the exception of *Nosema,* honey bee colonies experienced an increase in pathogen

342   prevalence and load over time with the highest levels occurring during the last sampling event in

343   March, following the seasonal trends of other time-course studies (Tentcheva et al., 2004;

344   Runckel et al., 2011). Peak incidences of these viruses occur in warmer months when

345   transmission is more likely to occur as a result of increased brood rearing (Chen & Siede, 2007)

346   and increased foraging (Singh et al., 2010) However, for BQCV and *Varroa*, our results indicate

347   that bees in the migratory conditions were affected differently compared to their stationary

348   counterparts.

349   The migratory colonies in our study returned from almond pollination with higher BQCV

350   loads compared to the stationary colonies but had converged to similar levels one month later

351   indicating that migratory conditions exacerbated BQCV infection but these effects were

352   transient. Colonies experience stress during transportation (Simone-Finstrom et al., 2016) which

353   impairs immunity (James & Xu, 2012) and promotes elevated levels of virus replication.

354   Pollinators of large monocultures experience a reduction in forage diversity (Decourtye, Mader

355   & Desneux, 2010; Colwell et al., 2017) which increases susceptibility to disease (Di Pasquale et

356   al., 2013). Exposure to agricultural chemicals adversely affects the insect immune response and

357   promotes replication of RNA viruses in bees (Di Prisco et al., 2013; Doublet et al., 2015). In

358   particular, higher BQCV titers are associated with exposure to organosilicone surfactant

359   adjuvants (OSS), a class of surfactants used to enhance the spread of the active ingredient (Fine,

360   Cox-Foster & Mullin, 2017). OSSs are heavily used in California almonds during the late

361   January to March bloom period when migratory colonies are present (Ciarlo et al., 2012; Mullin

362   et al., 2016). In addition to OSSs, bees involved in almond pollination may also be exposed to a

363   wide range of pesticides. In recent years, the use of insecticides, herbicides, and fungicides has

364   steadily increased in California almond crops (CDPR (California Department of Pesticide

365   Regulation) CalPIP, 2016). We did not measure pesticide or OSS exposure in our colonies and

366   are therefore cautious to speculate its role in the increased virus loads in our study. However, in

367   light of our results and previous work, we believe pesticide-pathogen interactions in migratory

368   colonies warrants further study.

369   Compared to stationary colonies, the migratory colonies had fewer FOB upon return from

370   California. The lower population size observed may be a result of forager die-off after the large

371   pollination event, as migratory bees have significantly shorter lifespans when compared to

372   stationary bees as a result of increased oxidative stress (Simone-Finstrom et al., 2016). In

373   addition, foragers could have been displaced during transit. As typical with migratory colonies,

374   our colonies were moved to holding yards before and after pollinating almonds. When colonies

375   are moved, foragers are forced to re-assess and re-learn their surroundings which can cause

376   significant loss and/or drifting of foragers (Nelson & Jay, 1989). Despite migratory colonies

377   returning with fewer numbers and remaining lower in FOB compared to stationary colonies, the

378   two groups experienced similar population growth rates during the month following the large

379   pollination event.

380   Upon return from California, mite prevalence and load in migratory colonies were similar

381   to their stationary counterparts. However, when sampled one month later, mite prevalence and

382   load in the stationary colonies had significantly increased, while mite prevalence and load in the

383   migratory colonies declined slightly, and was significantly lower than that in stationary colonies.

384   Since female mites must reproduce within the pupal cells of developing honey bees, mite

385   population growth is largely dependent on the availability of bee brood. Although we did not

386   measure brood size, adult bee population size is highly correlated with brood size of the previous

387   time step (Torres, Ricoy & Roybal, 2015) and mite population size (Martin, 1998; DeGrandi-

388   Hoffman et al., 2016) . Thus, the lower mite prevalence and load in migratory bees is likely, in

389   part, a reflection of the lower reproduction of these colonies. Additional unknown factors may be

390   influencing the lower mite loads in migratory colonies, as *Varroa* loads of the migratory and

391   stationary colonies showed different relationships with FOB over time. Results of the US

392   National Honey Bee Disease survey suggested that migratory beekeepers may treat with

393   acaricide more effectively and the mechanical motion of the truck during transportation helps to

394   dislodge mites from bees (Traynor et al., 2016b). Since our colonies returned from California

395   with similar mite prevalence and load as the stationary group, it is unlikely that the motion of the

396   truck had an impact. Additionally, we are confident that the difference in mites we saw during

397   the last sampling event was not due to beekeeper practices as mite treatments were standardized

398   across all groups.

399   Colonies exposed to migratory bees experienced a significantly greater increase in DWV

400   prevalence and load compared to isolated colonies one month after foraging alongside the

401   migratory colonies. *Varroa* loads could not explain this difference since exposed and isolated

402   colonies experienced similar *Varroa* loads throughout the study. The greater population size of

403   the exposed colonies in the last sampling event, could have increased dissemination of DWV.

404   However, isolated colonies had higher bee populations than the migratory colonies and we saw

405   no differences in DWV prevalence or load between those two groups. Previous studies found

406   that DWV was a good predictor of weaker colonies (Budge et al., 2015) and thus one would not

407   expect our results to simply be attributed to an increase in numbers and thus exposure. One

408   potential explanation is that the migratory bees returned from pollinating almonds with a more

409   virulent DWV strain that disseminated quickly in the exposed group as a result of their larger

410   colony size and higher *Varroa* population (Martin, 2002; Rosenkranz, Aumeier & Ziegelmann,

411   2010; Glenny et al., 2017). Using deep sequencing, viruses not previously found in honey bees

412   have been detected in migratory hives (Runckel et al., 2011) and recently, a more virulent

413   recombinant of DWV was found to replicate at high levels when transmitted by *Varroa* mites

414   (Ryabov et al., 2014). Despite this evidence, we remain cautious of speculating transmission of a

415   novel or more virulent strain.

Conclusions

417   Migratory bees are subjected to a myriad of stressors not experienced by their stationary

418   counterparts including transport, lower diversity of floral resources, exposure to bees from tens

419   of thousands of other colonies that may be diseased, and exposure to large quantities of

420   pesticides. The migratory conditions in our experiment encompassed all these components, and

421   thus we cannot attribute our results to a single or even an exact combination of causes.

422   Furthermore, our study, while novel in scope, was conducted over a relatively short time span

423   using a single set of migratory conditions and focused on a limited set of bee pathogens. Thus,

424   we are cautious to claim that our results are representative of migratory beekeeping, at large, but

425   do suggest that migratory conditions may exacerbate BQCV infections and lead to slower colony

426   growth. Future studies to examine the underlying mechanisms, individually and in concert, as

427   well as those that encompass colony health and additional pest and pathogens over a longer time

428   span will provide further insight.

429   A growing body of evidence suggests that pests and pathogens from managed bees are

430   spilling over into wild bee populations (Colla et al., 2006; Spiewok & Neumann, 2006;

431   Hoffmann, Pettis & Neumann, 2008; Otterstatter & Thomson, 2008; Singh et al., 2010;

432   Graystock et al., 2013; Levitt et al., 2013; Brown, 2017). Sympatric bumble bees and honey bees

433   are infected by the same strains of DWV (Fürst et al., 2014) and virus prevalence in honey bees

434   is a significant predictor of virus prevalence in bumble bees (McMahon et al., 2015). The higher

435   BQCV load we document in migratory bees could thus pose a risk to wild bees. It is also

436   possible that increased disease load as a consequence of migratory pollination could affect honey

437   bees in future years due to disease spillback from infected wild bees (Graystock, Goulson &

438   Hughes, 2015). Therefore, it is important to test whether wild bee populations have higher

439   disease prevalence in proximity to honey bee apiaries, particularly those with migratory

440   management practices.

441   According to recent forecasts, the US demand for commercial crop pollination services is

442   expected to rise, particularly for almond (USDA National Agricultural Statistics Service, 2017c).

443   Thus, understanding the effects of this current model of crop pollination on bees and identifying

444   where, when, and how to mitigate those effects are critical to the apiculture industry. Our work

445   suggests that some effects, while important, may be transitory. Thus, honey bees may be resilient

446   to some stressors imposed by migratory conditions and recuperation after large pollination events

447   is important to maintaining healthy migratory colonies.

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451

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Figure 1

Schematic of Experimental Design

Three sampling events occurred during the experiment. Three experimental groups (isolated stationary group, migratory group, and exposed group) were located in two separate apiaries in North Carolina throughout the experiment: the stationary yard (where all groups begin and the isolated stationary group remained for the duration of the experiment) and the exposed yard (where the exposed group was exposed to the migratory group). Dotted arrows show movement of colonies throughout the experiment. Between sampling events one and two, the migratory colonies were transported to California for almond pollination and back. Exposed colonies began in the stationary yard and were transferred to the exposed yard prior to sampling event two. Geographic distance between yards are specified in kilometers.

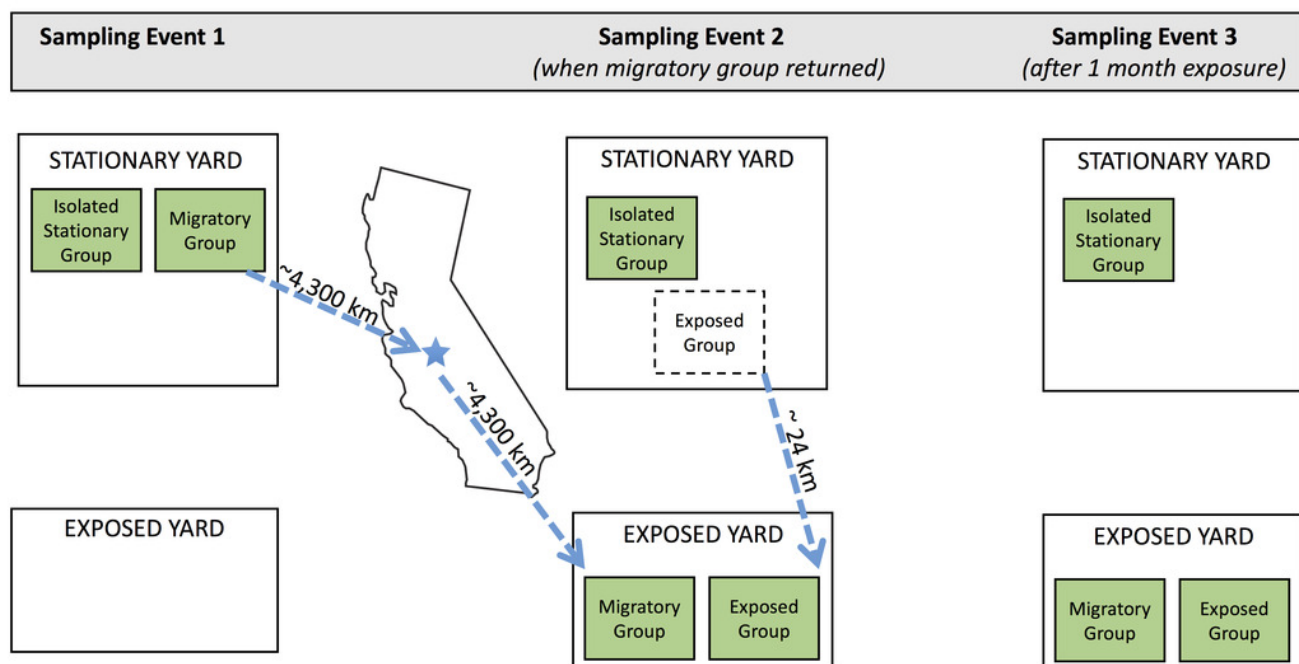



Figure 2

Pathogen community and colony health predicts treatment group membership

Linear combinations from discriminant analyses created from all pathogen variables (except BQCV prevalence) and frames of bees for exposed (black), migratory (red) and stationary/isolated (blue) colonies. Axes represent the percentage of between group variance explained.(A) Experiment 1 at sampling event two, migratory and stationary colonies were separated by LD1 while stationary and exposed colonies are clustered. B)Experiment 2 at sampling event three, after the exposed group had been allowed to forage alongside the migratory colonies, exposed and isolated were separated along LD2, while LD1 separated out migratory colonies.The significant PERMANOVA tests for both experiments corroborated the differences between group centroids. Circles represent 70% confidence intervals and are provided to visualize the centroids of each group.

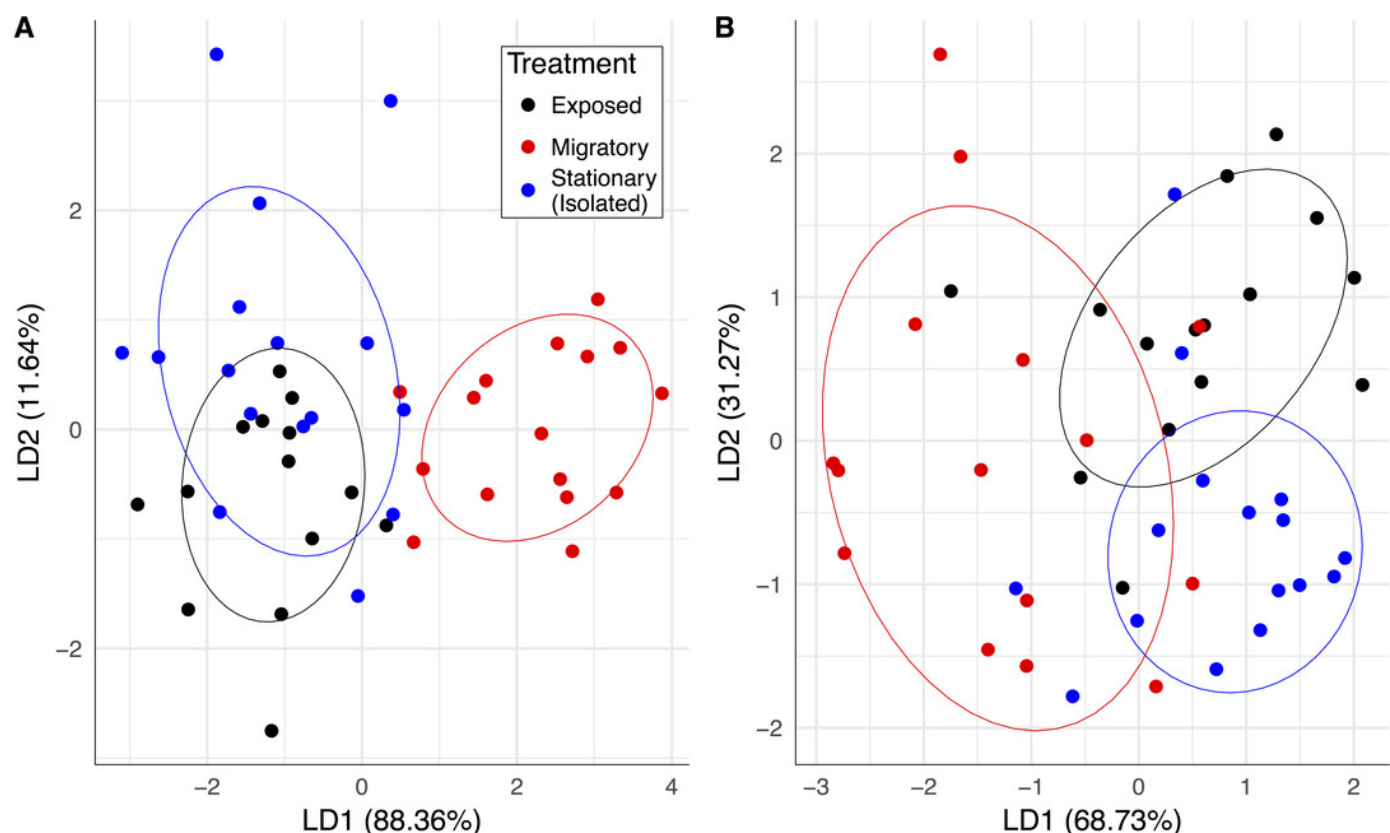
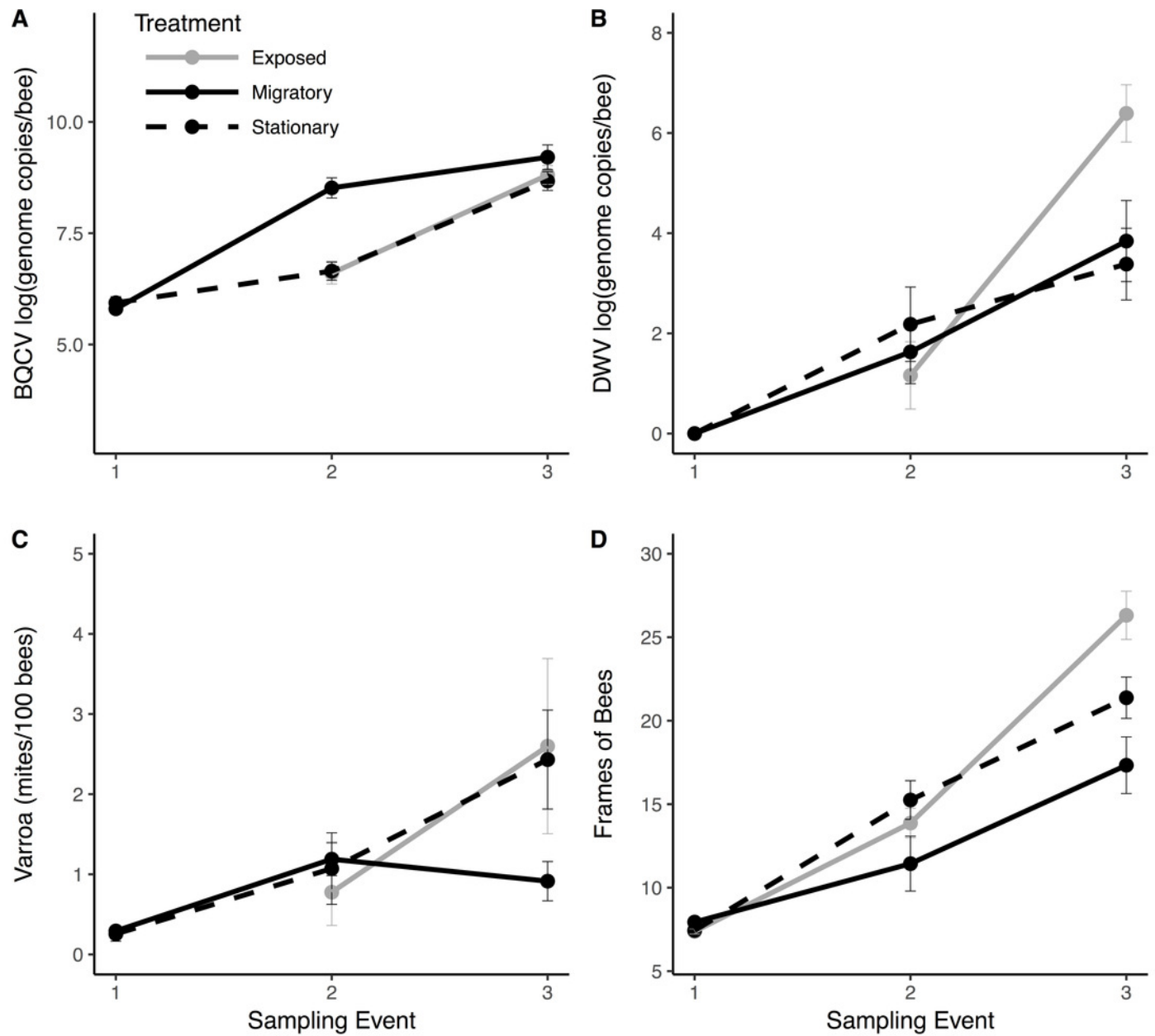



Figure 3

Pathogen and colony population metrics for treatment groups through time

Migratory (solid line) and stationary/isolated (dotted line) colonies were sampled at three time points and exposed (gray) colonies were sampled at two time points. Sampling event (1) occurred before migratory colonies were transported, (2) upon their return, and (3) one month after return. Panels show results for three pathogens and one health metric: (A) black queen cell virus (BQCV) in log genome copies per bee (B) deformed wing virus (DWV) in log genome copies per bee (C) *Varroa* load in mites per 100 bees and (D) Frames of bees (FOB), as a proxy for colony population. In Experiment 1: migratory verses stationary/isolated colonies, there was a significant effect of time for all measures. For BQCV, there was a significant effect of treatment. There was a significant time × treatment interaction for FOB and *Varroa.* In Experiment 2: exposed colonies verses stationary/isolated, there was a significant effect of time for each measure. For DWV, there was a significant time × treatment interaction. Bars represent standard errors.

Summary statistics for experiment 1: migratory verses stationary

DWV load, deformed wing virus load; DWV prev., deformed wing virus prevalence; BQCV load, black queen cell virus load; *Varroa* prev., *Varroa* prevalence; *Nosema* prev., *Nosema* prevalence; FOB, frames of bees. Prevalence is the percentage of colonies positive for a pathogen (DWV, *Nosema,* and *Varroa*). Virus load (DWV and BQCV) results for each colony are presented in average virus genome copies/bee. *Nosema* load is reported as average number of spores/bee and *Varroa* is reported as number of mites per 100 bees.

Variable	Effect	χ^2_1	P^a	Sig^b
DWV load	Treatment	0.004	0.9512	
	Time	39.328	<0.001	***
	Treatment:Time	0.1592	0.690	
DWV prev.	Treatment	0.067	0.796	
	Time	15.805	<0.001	***
	Treatment:Time	0.024	0.878	
BQCV load	Treatment	16.488	<0.001	***
	Time	187.235	<0.001	***
	Treatment:Time	2.229	0.135	
Varroa **load**	Treatment	0.413	0.520	
	Time	18.391	<0.001	***
	Treatment:Time	6.465	0.011	*
Varroa **prev.**	Treatment	1.290	0.256	
	Time	4.896	0.0270	*
	Treatment:Time	3.21	0.073	
Nosema **load**	Treatment	0.645	0.422	
	Time	30.855	<0.001	***
	Treatment:Time	0.280	0.596	
Nosema **prev.**	Treatment	0.007	0.931	
	Time	3.652	0.056	
	Treatment:Time	3.352	0.067	
FOB	Treatment	3.597	0.058	
	Time	152.838	<0.001	***
	Treatment:Time	5.651	0.0174	*

3   [a] Significance for all models was determined using Type II Wald Chi-Square tests.

4   [b] Asterisks represent level of significance.

Summary statistics for experiment 2: exposed verses isolated

DWV load, deformed wing virus load; DWV prev., deformed wing virus prevalence; BQCV load, black queen cell virus load; *Varroa* prev., *Varroa* prevalence; *Nosema* prev., *Nosema* prevalence; FOB, frames of bees. Prevalence is the percentage of colonies positive for a pathogen (DWV, *Nosema,* and *Varroa*). Virus load (DWV and BQCV) results for each colony are presented in average virus genome copies/bee. *Nosema* load is reported as average number of spores/bee and *Varroa* is reported as number of mites per 100 bees.

Variable	Effect	χ^2_1	P^a	Sig.^{b}
DWV load	Treatment	2.056	0.152	
	Time	23.510	<0.001	***
	Treatment:Time	9.229	0.002	**
DWV prev.	Treatment	0.025	0.874	
	Time	8.811	0.003	**
	Treatment:Time	4.945	0.026	*
BQCV load	Treatment	1.355	0.244	
	Time	58.001	<0.001	***
	Treatment:Time	0.054	0.816	
Varroa **load**	Treatment	0.471	0.493	
	Time	23.658	<0.001	***
	Treatment:Time	0.191	0.662	
Varroa **prev.**	Treatment	1.390	0.238	
	Time	10.129	0.001	**
	Treatment:Time	0.060	0.806	
Nosema **load**	Treatment	0.882	0.348	
	Time	37.926	<0.001	***
	Treatment:Time	0.260	0.610	
Nosema **prev.**	Treatment	0	0.1	
	Time	7.771	0.005	**
	Treatment:Time	0.004	0.950	
FOB	Treatment	1.899	0.168	
	Time	89.191	<0.001	***
	Treatment:Time	9.946	0.002	**

3   ^{a} Significance for all models was determined using Type II Wald Chi-Square tests.

4   ^{b}Asterisks represent level of significance.