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Presence and Prevalence of Viruses in Local and Migratory Honeybees (*Apis mellifera*) in Massachusetts▽

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Migratory and local bees in Massachusetts were analyzed for seven viruses. Three were detected: black queen cell virus (BQCV), deformed wing virus (DWV), and sacbrood virus (SBV). DWV was most common, followed closely by BQCV and then by SBV. BQCV and SBV were present at significantly higher rates in the migratory bees assayed, bringing into question the impact that these bees have on the health of local bee populations.

Fn1/AQ:A AQ: B The European honeybee, *Apis mellifera*, is found throughout the world and, as the principal pollinator of commercially important food crops, plays an important role in the global economy (10, 14). In Massachusetts, honeybees pollinate of a variety of economically significant crops, including apples, blueberries, and cranberries. As in other states, the demand for honeybee pollinators is met both by bees from local apiaries and by bees provided by commercial, migratory beekeepers, who transport bees across the country to meet pollination demands. This demand for bees during specific time windows, while crops are in bloom, often results in large numbers of hives arriving from different regions of the country to a small geographical location. For example, every year, an estimated 60% of all the commercial bee colonies in the United States are concentrated in a 500-mile stretch of California's Central Valley for use in the pollination of almond trees (26). Movement of bees in migratory hives to different areas of the country and the fact that hives from multiple locations may be placed at close proximity to one another put these bees at a higher risk of encountering pathogens than bees in local apiaries, which are maintained at a single locale, or moved only short distances.

In order to examine this possibility more closely, we assayed for the presence and prevalence of seven viruses in apparently healthy bees from local apiaries in Massachusetts and in bees brought into the state by migratory beekeepers. Honeybees are susceptible to a variety of pathogens, including *Nosema* spp., several species of fungi, bacteria, and as many as 18 different viruses (2, 3). The recent decline in managed European honeybee populations around the world, termed colony collapse disorder (CCD), has sparked interest in identifying factors affecting bee colony health (13). Although several pathogens have been found in bee colonies suffering from CCD, two of them are viruses: Israel acute paralysis virus (IAPV) and Kashmir bee virus (KBV). First reported in 2004 (20), IAPV has been identified as a significant indicator of this disease in honeybees (13) and appears to be an important, newly emerging pathogen (22).

Using reverse transcription-PCR (RT-PCR) on individual bees collected from migratory and locally managed beehives, we detected the presence of black queen cell virus (BQCV), deformed wing virus (DWV), and sacbrood virus (SBV) and determined the prevalence of these viruses in each hive. Migratory bees from three different populations were collected from their holding yard in Wareham, MA, in June 2008 (blue population, green population, and pink population), after having been used to pollinate cranberries in Massachusetts, blueberries in Maine, and almonds in California. Bees from a local, stationary hive at the UMass Cranberry Station in East Wareham, MA, were collected in July 2008 (State Bog I) and in October 2008 (State Bog II). Local bees were also collected from East Sandwich, MA, in June 2008 (E. Sandwich), and bees from an unmanaged hive in Plymouth, MA, were collected in October 2008 (Plymouth). TriReagent (MRC Gene, Inc.) was used to isolate total RNA from the collected bee samples, according to manufacturer's protocols. RT-PCR was performed with RT-PCR master mix (2×; USB Corporation) and previously reported primer sets specific to seven of the most prevalent bee viruses (Table 1). The primer set pairs T1 DWV and BQCV, from Chen et al. (9) and Benjeddou et al. (4), and SBV and KBV, from Chen et al. (9) and Siede et al. AQ: C (27), were initially used as a four-target multiplex reaction, according to Chen et al. (9). Amplification of each of the four targets was not detected until the appropriate pair of each primer set was used in the same reaction. All samples in this study were analyzed in this manner in order to reduce the number of reactions necessary to detect these viruses. Primer sets for ABPV (18), IAPV (Y. P. Chen, personal communication), CBPV (23), and KBV did not amplify any nucleic acids from the samples in this study. Positive controls from previously identified infected honeybee samples were included for all viruses except ABPV and CBPV. Reaction products were run on agarose gels to confirm base pair size, and the amplicons were sequenced using the ABI Prism Big Dye Terminator cycle sequencing ready reaction kit, version 1.1, on an ABI Prism 3130xl genetic analyzer (Perkin-Elmer Applied Biosystems). Sequence identity analysis was performed using the BLAST server (NCBI). Comparison between published viral

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TABLE 1. Primers used in this study

Primer	Position in the genome (nt)[a]	Product size (bp)	Reference or source
Acute bee paralysis virus ABPV-1 (5′-AGCCACTATGTGCTATCGTAT-3′) ABPV-2 (5′-ATGGTGACCTCTGTGTCATTA-3′)	34–240 (5′ UTR)	207	18
BQCV BQCV-mvi-F (5′-TGGTCAGCTCCCACTACCTTAAAC-3′) BQCV-mvi-R (5′-GCAACAAGAAGAAACGTAAACCAC-3′)	7850–8550 (Structural polyprotein)	700	4
Chronic bee paralysis virus CBPV-F (5′-AGTTGTCATGGTTAACAGGATACGAG-3′) CBPV-R (5′-TCTAATCTTAGCACGAAAGCCGAG-3′)	2580–3034 (Nonstructural polyprotein RdRp)	455	25
DWV DWV-mvi-F (5′-CTTACTCTGCCGTCGCCCA-3′) DWV-mvi-R (5′-CCGTTAGGAACTCATTATCGCG-3′)	1171–1365 (Structural polyprotein)	194	9
IAPV IAPV-j-F (5′-GCGGAGAATATAAGGCTCAG- 3′) IAPV-j-R (5′-CTTGCAAGATAAGAAAGGGGG-3′)	23–609 (5′ UTR)	587	Y. P. Chen, personal communication
KBV KBV-mvi-F (5′-GATGAACGTCGACCTATTGA-3′) KBV-mvi-R (5′-TGTGGGTTGGCTATGAGTCA-3′)	5405–5820 (Nonstructural polyprotein RdRp)	415	29
SBV SBV-mvi-F (5′-GCTGAGGTAGGATCTTTGCGT-3′) SBV-mvi-R (5′-TCATCATCTTCACCATCCGA-3′)	4957–5781 (Nonstructural polyprotein)	824	9

[a] UTR, untranslated region.

sequences in GenBank and individual PCR products resulted in sequence identities of 99% for BQCV, 98% for DWV, and 94% for SBV.

Because evidence of a virus detected in bees was recorded as presence or absence, the mathematical model for the distribution of specific viruses is binomial and multinomial when more than one virus coinfecting the host bee is considered. Therefore, nominal logistic regression was used to assess differences in the infection levels of individual viruses between local and migratory colonies (1). Ordinal logistic regression was used to test differences in multiple virus infections (1). Bees were coded as no viral infection, infection by a single virus, or infection by two or even three viruses. A second-tier analysis was then conducted with nominal or logistic regression to assess differences in infection between colonies within local or migratory treatments because of the nested experimental design (bees within populations of colonies and populations of colonies within migratory treatment versus those within local treatment) (12). Pairwise comparisons were conducted to determine the specific number and pattern of differences between the various populations in each of the two migratory treatment groups (12). The results of this analysis for honeybees from local and migratory populations infected with each of the viruses individually and in combination are presented in Table 2 and discussed below.

BQCV, DWV, and SBV were the only viruses detected in this survey and were each present in both local and migratory bee populations in Massachusetts. Of the over 300 bees examined in this study, only five were found not to be infected with at least one of these viruses. The two most common viruses found in both migratory and local bees were BQCV and DWV. DWV was identified in 98% of the bees from local hives and in 72% of bees in migratory hives, and BQCV was identified in 60% and 92%, respectively. All three migratory hives assayed shared this trend of having a high rate of infection with DWV and an even higher rate of infection with BQCV. The local hives examined followed the trend of having high rates of infection with both DWV and BQCV. However, with the exception of bees from E. Sandwich, MA, in which the infection rate for both BQCV and DWV was over 95%, the infection rate of DWV in local bees was always higher than that of BQCV. The differences in prevalence of these two viruses between local and migratory bees may be related to the different methods used for pathogen or parasite control, including methods used to control the parasitic mite *Varroa destructor* Anderson & Trueman, which is known to be an important vector of DWV in honeybee colonies (7, 29, 30). The extreme prevalence of DWV is not unexpected, as it is probably the most widespread of the honeybee viruses (6, 10). Furthermore, a survey of queens in the United States showed BQCV and DWV to be the most prevalent viruses in these bees (10). Although the symptomatic wing deformities and abdominal shortening associated with DWV pathology are quite visibly noticeable when present (16), none were observed in the bees in this study. This is not surprising, as only a few bees in a colony show wing deformities, even in those hives that are severely infested with *V. destructor* (29).

Migratory bees collected for this study showed a much higher prevalence of SBV (16%) than did local bees (<1%). Only one SBV-infected bee was found in all four local hives that were examined. The infrequent occurrence of SBV in local bees and the presence of this virus in almost one-fifth of

TABLE 2. Percentage of virus-positive honeybees from migratory and local beehives collected from Massachusetts[a]

Population	*n*	% Honeybees positive for:						
		Single infection			Dual infection			Triple infection (BQCV DWV SBV)
		BQCV	DWV	SBV	BQCV DWV	BQCV SBV	DWV SBV	
Migratory hive populations								
Blue	48	93.8	66.7	14.6	77.1	6.3	0	8.3
Green	48	95.8	77.1	22.9	54.2	4.2	0	18.8
Pink	48	85.4	70.8	10.4	50.0	2.1	0	8.3
Avg	**144**	**91.7**	**71.5**	**15.9**	**60.4**	**4.2**	**0**	**11.8**
Local hive populations								
E. Sandwich	20	95.0	100	0	95.0	0	0	0
State Bog I	48	68.7	93.8	0	68.8	0	0	0
State Bog II	48	77.0	100	0	77.1	0	0	0
Plymouth	48	20.8	100	2.1	20.8	0	2.1	0
Avg	**164**	**60.3**	**98.2**	**0.6**	**61**	**0**	**0.6**	**0**

[a] Values in boldface type are the averages. *n* indicates the number of honeybees in the sample.

the migratory bees suggest that migratory bees could potentially act as carriers of this virus and serve to introduce SBV into local bee populations in Massachusetts.

Bees infected with multiple viruses were common in both local and migratory populations, with more than 60% of all the bees examined being infected with more than one virus. The most common pair of viruses constituting a dual infection was BQCV and DWV, and these were the two most common viruses found in bees overall. It is interesting that all of the bees from one local hive, E. Sandwich, that were infected with DWV were also infected with BQCV. For all the other hives, the percentage of bees infected with both of these viruses (BQCV and DWV) was never greater than 77% and was as low as 21%. While instances of multiple virus infections have been reported many times, there is still much to ascertain about their significance in hive health. The details of the immunological effects in bees simultaneously infected with more than one virus are unknown. It is also unknown if bees experiencing multiple virus infections could facilitate an environment favorable for recombination between viruses, possibly resulting in a new virus (9).

It is noteworthy that more variation in virus prevalence was present between the local bee populations. The migratory bees were more consistently infected and had a significantly higher prevalence of triple infections. This may be due to the differences in both exposure to pathogens that migratory and local bees experience and overall fitness of the hives as related to stress.

Although BQCV was found to be more prevalent in migratory bee populations, it was also common in the local populations, suggesting that migratory bees are no more likely to spread BQCV than are the local populations themselves. However, the increased prevalence of SBV in migratory bees suggests that these hives may be a source of this virus, potentially posing a threat to the health of local populations.

As the practice of commercial, migratory beekeeping remains a necessity for viable crop production in much of the world, the potential impacts of this practice on pathogen transmission and bee colony health will need to be identified. The viral status of local, stationary, domesticated *A. mellifera* populations around the world, as described in several reports, can be useful in determining the impact of migratory beekeeping (5, 11, 15, 19, 21, 24, 25, 28, 29). Due to differences in methods of sampling and viruses examined, it is difficult to make comparisons between reports on the prevalence of viruses in bee populations around the world; however, some general trends are apparent. The first is that DWV, the most prevalent virus found in bees in Massachusetts, is reported in nearly every survey of bee viruses (10, 19, 21, 25, 28, 29). The ubiquitous nature of DWV in honeybee populations around the world may be a result of its association with other invertebrate hosts, such as bumble bees, *Bombus* spp., (17) and *Varroa* (6, 11). BQCV, which was the second most prevalent virus found in our study, has been reported in Spain, France, Hungary, Austria, and Brazil and in Denmark at a very low rate (5, 15, 19, 21, 28, 29) but was not found in surveys of honeybees from Thailand (24). SBV, which was found to be prevalent in migratory bees but not in local Massachusetts bees, was also prevalent in bees from Denmark, France, and Austria (5, 21, 29) and absent or present at a very low rate in bees from Thailand, Hungary, and Brazil (15, 25, 28). Two other viruses that were not detected in Massachusetts bees, ABPV and CBPV, have been found in fairly high levels in bees from other countries, with ABPV being found in bees from Denmark, France, Hungary, Austria, Brazil, and Thailand (5, 15, 21, 24, 28, 29) and CBPV in bees surveyed in France, Denmark, and Austria (5, 21, 29). The lack of ABPV-positive samples in this study agrees with another survey of bees done in the United States, as it was not found in any tissues of the queen bees analyzed (11).

The reasons for differences in prevalence of bee viruses worldwide are not fully known and may be related to bee management and propagation practices or possibly the presence of alternative hosts or vectors for these viruses (8, 7, 17, 29, 30). Also, some variation in prevalence is undoubtedly due to different methods of honeybee sampling and the analysis of results. It is important for the future of local and migratory honeybee colony health to continue to monitor and control these viruses and the diseases they cause as well as to identify newly emerging viruses, like IAPV, so that future problems

with honeybees, like CCD, can be avoided or limited in their spread within bee populations.

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