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Short Communication

First detection of *Apis mellifera* filamentous virus in *Apis cerana cerana* in China

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ABSTRACT

Although many honey bee RNA viruses have been correlated with colony declines, little is known regarding the potential role of DNA viruses. Here, we examined seemingly healthy and crawling bee samples from China using PCR to identify whether *Apis mellifera* filamentous virus (AmFV) was present in *A. cerana cerana*. The highest AmFV infection percentage among Chinese provinces occurred in crawling bees from Gansu province (85.48%), and the lowest was in bees from Beijing (31.58%). A phylogenetic analysis showed that the Chinese isolate of AmFV exhibited a high genetic similarity with isolates from Belgium, Switzerland and USA. This is the first report of AmFV infections in Chinese *A. cerana cerana* populations.

1. Introduction

Apis cerana cerana is one of the most important pollinators in China and has been domesticated for more than 2000 years (Su and Chen, 2009). *A. cerana cerana* possesses several behavioral and physiological characteristics that are not present in *Apis mellifera*, such as resistance to the ectoparasitic mite, *Varroa destructor*, and the ability to forage under low temperatures (Chen et al., 2015).

In particular, *A. cerana cerana* plays a vital role in the pollination of crops and the natural vegetation of mountain areas, and can also utilize scattered nectar resources (Yang, 2005). However, the population numbers of *A. cerana cerana* have been decreasing in recent years. In addition, our research group has recently obtained reports from beekeepers that have described this decrease and the large numbers of bees crawling at the entrance of *A. cerana cerana* hives. These reports have not previously attracted much attention from scientists focused on honey bee pathology. Although there are several threats to *A. cerana cerana*, little is known regarding honey bee viruses because most viruses primarily cause asymptomatic infections. So far, the only characterized RNA virus bee pathogen, which could pose a deadly threat to *A. cerana cerana*, is Chinese sacbrood virus (CSBV) (Ma et al., 2011). In addition, most characterized bee viruses are RNA viruses. However, a DNA virus, the *A. mellifera* filamentous virus (AmFV), was recently found to be related to a decrease in honey bee colonies during the spring in Switzerland (Hartmann et al., 2015). Subsequently, the AmFV genome was published (Gauthier et al., 2015). Based on the link between AmFV and the bee crawling observed during the spring, we elected to evaluate for the presence of AmFV, which has never been described in China. We examined samples of *A. cerana cerana* from five provinces to determine if AmFV could be detected in this important Chinese bee species.

2. Materials and methods

2.1. Samples

Samples of crawling and seemingly healthy honey bees were collected from 228 colonies in total from Zhejiang (25 colonies from 3 apiaries), Henan (46 colonies from 5 apiaries), Liaoning (57 colonies from 6 apiaries), Beijing (38 colonies from 4 apiaries) and Gansu (62 colonies from 6 apiaries) provinces in China during the spring of 2015. We collected random samples of crawling and seemingly healthy bees outside and inside of each colony. We collected crawling bees because bees infected by AmFV have frequently been found in front of the beehive (Clark, 1978). We collected all samples on dry ice and stored them at −80 °C until use.

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2.2. DNA and RNA extraction

Honey bee samples collected at the hive entrance (approximately 30 crawling bees) and from the inner lid (approximately 30 seemingly healthy bees) from each colony were homogenized using a Tissueprep® homogenizer (Getting Scientific Instrument Ltd., Beijing, China) in a 15 mL sterile tube, using the Genomic DNA Kit (TianGen Biotech Co., Ltd., Beijing, China) and RNA Purification Kit (Promega Corp., Madison, WI, USA) for DNA and RNA extraction, respectively, according to the manufacturer's instructions. We also evaluated for the presence of CSBV and sacbrood virus (SBV), which are currently believed to represent the most significant threats to *A. cerana cerana* (Ma et al., 2011). Total DNA and RNA were dissolved in 20 μL of sterile water and stored at −20 °C until use. The synthesis of cDNA was performed using an RT-PCR Kit (Promega Corp., Madison, WI, USA).

2.3. Molecular detection

PCR amplification was carried out in a 20 μL volume. This PCR mixture contained template DNA or cDNA, 2 × GoTaq Mix (Promega Corp., Madison, WI, USA), and primers as described in Table 1 (Ma et al., 2011; Hou et al., 2014; Hartmann et al., 2015). Amplification was run on an ABI PCR machine (Gene Company Ltd., Hong Kong, China) for 3 min at 94 °C; followed by 30 successive cycles of 30 s at 94 °C, 30 s at 55 °C and 72 °C for 1 min; and a final extension of 10 min at 72 °C. The PCR products were examined by electrophoresis on a 1.2% agarose gel containing Gold View II nucleic acid stain (SBS Genetech Corp. Ltd., Beijing, China). Then, the PCR products were purified and sequenced, and a phylogenetic analysis was performed based on the amino acid sequence using the neighbor-joining likelihood method as run on MEGA 6.0 software and 1000 bootstrap replicates. The obtained sequences were assembled using the Vector NTI Advance 11 software (Thermo Fisher Scientific Inc., Waltham, MA, USA). A chi-square test was performed using Sigmaplot 12.0 software (Systat Software Inc., San Jose, CA, USA).

Table 1
Primers used in the present study.

Virus	Primer sequences (5′–3′)	References
AmFV	Forward: CAGAGAATTCGGTTTTTGTGAGTG Reverse: CATGGTGGCCAAGTCTTGCT	Hartmann et al. (2015)
SBV	Forward: ATA TAC GGT GCG AGA ACT GC Reverse: CTC GGT AAT AAC GCC ACT GT	Hou et al. (2014)
CSBV	Forward: CCTGGGAAGTTTGCTAGTATTTACG Reverse: CCTATCACATCCATCTGGGTCAG	Ma et al. (2011)

3. Results and discussion

We conducted a PCR analysis with seemingly healthy and crawling bees samples collected from inside and outside bee hives. Among the three viruses evaluated, CSBV and SBV were only detected in three provinces. CSBV was found in seemingly healthy bees from Zhejiang and Gansu provinces, while SBV was detected in seemingly healthy bees from Henan province. CSBV and SBV were not detected in any crawling bees from any of sampled provinces. Unexpectedly, AmFV was found in all of the colonies, in both crawling and seemingly healthy honey bee samples. However, AmFV was not detected in the negative control (Fig. 1A). The infection percentage among crawling bees from Gansu ($\chi^2 = 9.86$, $p < 0.01$, df = 1) was significantly higher than those of the other four provinces (Zhejiang, $\chi^2 = 0.88$, $p > 0.05$, df = 1; Henan, $\chi^2 = 2.4$, $p > 0.05$, df = 1; Liaoning, $\chi^2 = 0.15$, $p > 0.05$, df = 1; Beijing, $\chi^2 = 2.6$, $p > 0.05$, df = 1). Crawling bee samples collected in Gansu province displayed the highest infection percentage (85.48%), and the lowest infection percentage was detected in Beijing (31.58%); (Fig. 1B). By contrast, the highest infection percentage of seemingly healthy samples occurred in Henan province (54.35%), and the lowest percentage was in Beijing (10.53%).

The segment amplified with the AmFV primer-pair was sequenced, and a 551-bp fragment was identified. The nucleic acid sequence (GenBank accession number: KU047954) was aligned to the GenBank database, and the highest identity (98%) was found with sequences of AmFV from Switzerland (Accession No. JF304814) and Belgium (Accession No. KJ685944), which encode the same amino acids. Then, we performed a phylogenetic analysis based on the partial of amino acid sequences of BroN obtained from the current cloned AmFV and the NCBI database.

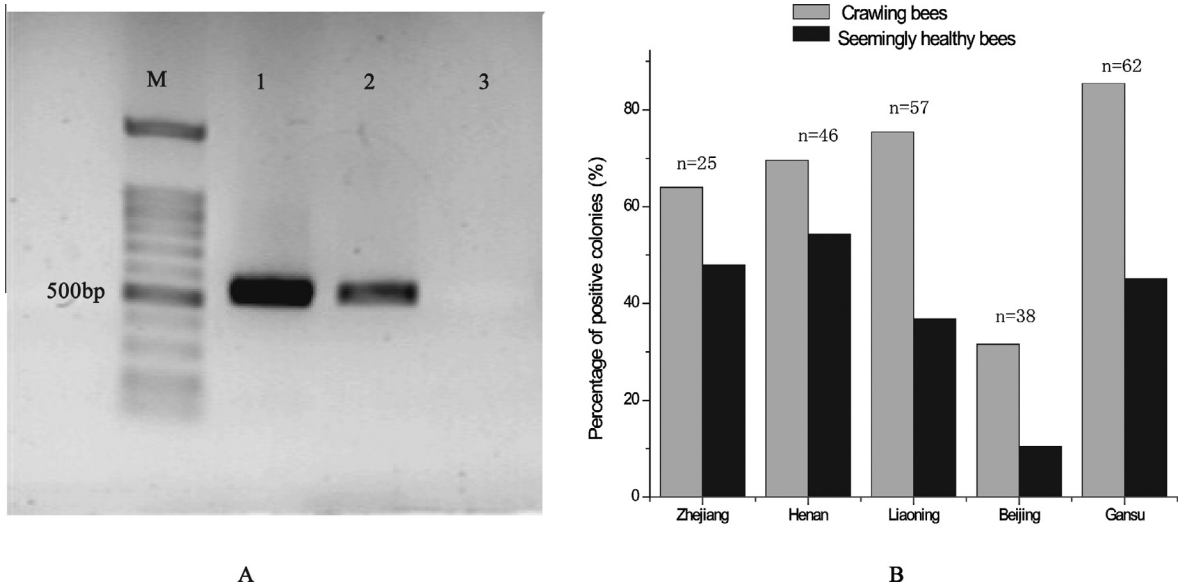

Fig. 1. (A) Detection of AmFV in crawling and seemingly healthy bee samples. M, molecular marker; 1, crawling bees; 2, seemingly healthy bees; 3, negative controls. (B) The percentage of honey bee colonies from five Chinese provinces infected by AmFV infection. n indicates the numbers of detected colonies in each province.