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Short Communication

Widespread occurrence of honey bee pathogens in solitary bees

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ABSTRACT

Solitary bees and honey bees from a neighbouring apiary were screened for a broad set of putative pathogens including protists, fungi, spiroplasmas and viruses. Most sampled bees appeared to be infected with multiple parasites. Interestingly, viruses exclusively known from honey bees such as *Apis mellifera* Filamentous Virus and *Varroa destructor* Macula-like Virus were also discovered in solitary bees. A microsporidium found in *Andrena vaga* showed most resemblance to *Nosema thomsoni*. Our results suggest that bee hives represent a putative source of pathogens for other pollinators. Similarly, solitary bees may act as a reservoir of honey bee pathogens.

1. Introduction

There is a long tradition of studying the pathogens of the Western honey bee (*Apis mellifera*) and the list of honey bee pathogens has been expanded significantly from the sixties on. It became recently evident that common honey bee pathogens such as Deformed Wing Virus (DWV) can infect other bees as well (Furst et al., 2014; Levitt et al., 2013). Although several macroparasites of wild bees are well known (Westrich, 1990), reports on their microparasites are rather scarce. The few known solitary bee-specific parasites are fungi, including *Ascosphaera* spp. (Wynns et al., 2013) and *Antonospora scoticae* (Fries et al., 1999).

The aim of this study was to investigate whether solitary bees sampled nearby an apiary harbour some of the known or recently discovered honey bee pathogens (Runckel et al., 2011). We screened for a broad set of parasitic micro-organisms, including fungi, protists, spiroplasmas and viruses.

2. Material and methods

2.1. Sample collection

For each species, three pooled samples of 10 bees were collected in 2012 at campus Sterre of Ghent University. *Osmia bicornis*, *Osmia cornuta* and *Heriades truncorum* were sampled at a bee hotel, close to an apiary. *Andrena vaga* and *Andrena ventralis* (only one sample of 10 bees) were caught nearby their nest aggregations. These species were identified using suitable keys (Scheuchl, 1996; Schmid-Egger and Scheuchl, 1997). Three different honey bee colonies were simultaneously (within one week) sampled at the neighbouring apiary.

2.2. RNA and DNA extraction

Ten bees were homogenised in 5 ml PBS in the presence of glass beads. Total RNA was extracted from 100 µl supernatant using the RNeasy Lipid Tissue Kit (Qiagen). Using random hexamer primers, 1 µg RNA was retro-transcribed with the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). DNA was extracted from 120 µl supernatant using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions for animal tissues.

2.3. PCR and BeeDoctor analysis

All PCR reaction mixtures contained: 2 µM of each primer (Table A1); 1.0 mM $MgCl_2$; 0.2 mM dNTPs; 1.25 U Hotstar Taq DNA polymerase (Qiagen) and 1 µl cDNA or 3 µl DNA product (*Apis mellifera* Filamentous Virus (AmFV) and *Ascosphaera* spp. detection) using described PCR cycles (Table A1). For our developed primers we used: 94 °C for 15 min; [94 °C for 30 s, 50 °C (*Crithidia* spp. cytochrome *b*) or 55 °C (LSV Orf1) for 30 s, 72 °C for 1 min] 35 cycles, 72 °C for 10 min. Positive and negative controls were always included. Amplicons intended for Genbank submission were amplified with Hotstar High Fidelity Taq DNA polymerase

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Table 1
Summary of pathogens detected in honey bees and solitary bees.

Sample	Date	Virus	*Crithidia* spp.	*Neogregarinida*	*Nosema* spp.	*Ascosphaera* spp.	*Spiroplasma* spp.
Hive 3–1	4 April 2012	ALPV, BQCV, LSV, AmFV	–	*A. bombi*	*N. apis, N. ceranae*	–	–
Hive 5–1	4 April 2012	ALPV, BQCV, LSV, AmFV	*C. mellificae*	*A. bombi*	*N. apis, N. ceranae*	–	–
Hive 10–1	4 April 2012	ALPV, BQCV, LSV, AmFV	*C. mellificae*	*A. bombi*	*N. ceranae*	–	–
O. cornuta 1	4 April 2012	BQCV, LSV, AmFV, VdMLV	–	*A. bombi*	*N. ceranae*	+	–
Hive 3–2	16 April 2012	ALPV, BQCV, LSV, AmFV	*C. mellificae*	*A. bombi*	*N. apis, N. ceranae*	*A. apis*	–
Hive 5–2	16 April 2012	ALPV, BQCV, LSV, AmFV	*C. mellificae*	*A. bombi*	*N. ceranae*	*A. apis*	–
Hive 10–2	16 April 2012	ALPV, BQCV, LSV, AmFV	*C. mellificae*	*A. bombi*	*N. ceranae*	*A. apis*	–
O. cornuta 2	16 April 2012	BQCV, LSV, AmFV, VdMLV	–	*A. bombi*	*N. ceranae*	*Ascosphaera* spp.	–
Hive 3–3	23 April 2012	ALPV, BQCV, LSV, AmFV	–	*A. bombi*	*N. apis, N. ceranae*	*A. apis*	–
Hive 5–3	23 April 2012	ALPV, BQCV, LSV, AmFV	*C. mellificae*	*A. bombi*	*N. ceranae*	*A. apis*	–
Hive 10–3	23 April 2012	ALPV, BQCV, LSV, AmFV	*C. mellificae*	*A. bombi*	*N. ceranae*	*A. apis*	–
O. cornuta 3	24 April 2012	BQCV, LSV, AmFV, VdMLV	–	*A. bombi*	*N. ceranae*	*Ascosphaera* spp.	–
A. vaga 1	24 April 2012	BQCV, LSV, AmFV	*C. bombi*	*A. bombi*	*N. thomsoni*	–	–
A. ventralis	24 April 2012	LSV, AmFV	–	*A. bombi*	*N. ceranae*	–	–
Hive 3–4	15 May 2012	ALPV, BQCV, LSV, AmFV	–	*A. bombi*	*N. apis, N. ceranae*	*A. apis*	–
Hive 5–4	15 May 2012	ALPV, BQCV, LSV, AmFV	–	*A. bombi*	*N. ceranae*	–	–
Hive 10–4	15 May 2012	ALPV, BQCV, LSV, AmFV	*C. mellificae*	*A. bombi*	*N. ceranae*	*A. apis*	–
A. vaga 2	8 May 2012	LSV, SBV, AmFV	*C. bombi*	*A. bombi*	*N. thomsoni*	–	–
A. vaga 3	8 May 2012	LSV, AmFV	*C. bombi*	*A. bombi*	*N. thomsoni*	–	
Hive 3–5	31 May 2012	ALPV, BQCV, DWV, LSV, VdMLV, AmFV	*C. mellificae*	*A. bombi*	*N. ceranae*	–	*S. melliferum*
Hive 5–5	31 May 2012	ALPV, BQCV, DWV, LSV, VdMLV, AmFV	*C. mellificae*	*A. bombi*	*N. ceranae*	–	–
Hive 10–5	31 May 2012	BQCV, DWV, LSV, VdMLV, AmFV	*C. mellificae*	*A. bombi*	*N. ceranae*	–	–
O. bicornis 1	30 May 2012	DWV, LSV, AmFV, VdMLV	*C. bombi*	*A. bombi*	*N. ceranae*	*Ascosphaera* spp.	*S. melliferum*
O. bicornis 2	30 May 2012	DWV, LSV, AmFV, VdMLV	*C. bombi*	*A. bombi*	*N. ceranae*	*Ascosphaera* spp.	*S. apis*
O. bicornis 3	30 May 2012	DWV, LSV, AmFV, VdMLV	*C. bombi*	*A. bombi*	*N. ceranae*	*Ascosphaera* spp.	*S. melliferum*
Hive 3–6	9 July 2012	ALPV, BQCV, DWV, LSV, VdMLV, AmFV	*C. mellificae*	*A. bombi*	*N. ceranae*	–	*S. melliferum*
Hive 5–6	9 July 2012	DWV, LSV, VdMLV, AmFV	*C. mellificae*	*A. bombi*	*N. ceranae*	*A. apis*	*S. melliferum*
Hive 10–6	9 July 2012	ALPV, BQCV, DWV, LSV, VdMLV, AmFV	*C. mellificae*	*A. bombi*	*N. ceranae*	–	*S. melliferum*
H. truncorum 1	5 July 2012	BQCV	–	–	*N. ceranae*	–	–
H. truncorum 2	5 July 2012	–	–	–	*N. ceranae*	–	–
H. truncorum 3	5 July 2012	BQCV	–	*A. bombi*	*N. ceranae*	–	–

Hive 3, 5 and 10: hive identification number, ALPV: Aphid Lethal Paralysis Virus, *A. vaga*: *Andrena vaga*, *A. ventralis*: *Andrena ventralis*, BQCV: Black Queen Cell Virus, *C. bombi*: *Crithidia bombi*, *C. mellificae*: *Crithidia mellificae*, DWV: Deformed Wing Virus, *H. truncorum*: *Heriades truncorum*, LSV: *Lake Sinai Virus*, *N. apis*: *Nosema apis*, *N. ceranae*: *Nosema ceranae*, *N. thomsoni*: *Nosema thomsoni*, *O. bicornis*: *Osmia bicornis*, *O. cornuta*: *Osmia cornuta*, SBV: Sacbrood Virus, VdMLV: *Varroa destructor* Macula-like Virus.

(Qiagen). PCR products were electrophoresed using 1.4% agarose gels, stained with ethidium bromide and visualised under UV light.

Honey bee samples (1 μl RNA) were screened using the BeeDoctor tool, capable of detecting actin (honey bee control gene), Acute Bee Paralysis Virus (ABPV), BQCV, Chronic Bee Paralysis Virus (CBPV), DWV, SacBrood Virus (SBV) and Slow Bee Paralysis Virus (SBPV) (De Smet et al., 2012). ABPV probes amplify ABPV, Israeli Acute Paralysis Virus and Kashmir Bee Virus; DWV probes amplify DWV, Kakugo Virus and *Varroa destructor* Virus-1 (VDV-1). Solitary bees were analysed for these viruses using RT-PCR. Other viruses (Aphid Lethal Paralysis Virus strain Brookings (ALPV), AmFV, Big Sioux River Virus (BSRV), Lake Sinai Virus (LSV), Tobacco Ringspot Virus (TRSV) and *Varroa destructor* Macula-like Virus (VdMLV), bacteria (*Spiroplasma* spp.), fungi (*Ascosphaera* spp. and *Nosema* spp.) and protists (*Apicystis bombi* and *Crithidia* spp.) were screened by PCR. The cytochrome *c* oxidase subunit I gene was used as control gene for the solitary bees.

2.4. Sequence analysis

Amplicons were cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen). Plasmids and PCR products were sequenced using gene-specific or M13 primers. Sequences are deposited as AB859946-AB859948 (DWV), AB860145-AB860146 (*Crithidia* spp.), AB859949-AB859952 (VdMLV), HG764796-HG764797 (BQCV), HG764798-HG764799 (SBV), KF768348-KF68351 (LSV), KJ685944 (AmFV), KJ685945-KJ685947 (*Ascosphaera* spp.).

Phylogenetic trees were inferred via maximum likelihood (ML) using PhyML 3.0 (Guindon et al., 2010) with the Le-Gascuel (LG) amino acid substitution model (Le and Gascuel, 2008) and approximate likelihood ratio test non-parametric branch support based on a Shimodaira-Hasegawa-like (aLRT SH-like) procedure (Anisimova and Gascuel, 2006).

3. Results and discussion

We were able to demonstrate the presence of AmFV in all samples. Most other viruses (BQCV, DWV, SBV and VdMLV) were detected both in honey bees and in a smaller subset of solitary bees (see Table 1). ALPV was detected in honey bee samples only, but the viruses ABPV, BSRV, CBPV, SBPV and TRSV were not discovered at all. Protists and fungi appeared to be pervasive, whereas spiroplasmas were found only scarcely.

The discovered AmFV sequences were mutually identical and highly similar to Baculovirus sequences from Swiss bees (Genbank: JF304814) and *V. destructor* mites (Cornman et al., 2010) (Fig. A.1). Further, amplicons of the ribonucleotide reductase small subunit and thymidylate synthase were identical to those found in *V. destructor* (Genbank: GU980896-GU980897). The LSV strains found in honey bees, *A. vaga*, *O. bicornis* and *O. cornuta* were almost identical (Genbank: KF768348-KF68350). However, a deviating LSV strain (Genbank: KF768351) was detected both in *A. ventralis* and in some honey bees. Their Orf1 sequences showed only 76.7% amino acid similarity with each other. DWV was detected in all honey bee hives and in *O. bicornis*. Their 5′ untranslated region (UTR) was equal to published DWV sequences. Although the 5′ UTR amplicons appeared mutually identical, phylogenetic analysis of the DWV L protein resulted in two divergent strains (Fig. 1). Strain e3 (Genbank: AB859948), only detected in honey bees,