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SCIENCE DIRECT
Journal of Invertebrate Pathology 91 (2006) 61–63
Journal of INVERTEBRATE PATHOLOGY
www.elsevier.com/locate/yjipa

Short communication

Detection of *Deformed wing virus*, a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities

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Received 6 July 2005; accepted 4 October 2005
Available online 21 November 2005

Abstract

Honey bees (*Apis mellifera*) productively infected with *Deformed wing virus* (DWV) through *Varroa destructor* (*V. destructor*) during pupal stages develop into adults showing wing and other morphological deformities. Here, we report for the first time the occurrence of bumble bees (*Bombus terrestris*, *Bombus pascuorum*) exhibiting wing deformities resembling those seen in clinically DWV-infected honey bees. Using specific RT-PCR protocols for the detection of DWV followed by sequencing of the PCR products we could demonstrate that the bumble bees were indeed infected with DWV. Since such deformed bumble bees are not viable DWV infection may pose a serious threat to bumble bee populations.

Keywords: DWV; Honey bee; Bumble bee

Deformed wing virus (DWV) is a honey bee pathogen which, like other honey bee viruses, generally persists as an inapparent infection. Outbreaks of clinical DWV infections are characterized by the occurrence of morphologically abnormal bees and are invariably associated with infestation by the parasitic mite *Varroa destructor* (Ball and Allen, 1988; Martin et al., 1998; Martin, 2001; Nordström, 2003). Morphological abnormalities include vestigial or crumpled wings, shortened, bloated abdomens, and discoloration. DWV has become one of the major virus threats to the honey bee industry due to its synergism with *V. destructor* (Martin, 2001), which acts as an inducer and vector of the virus (Ball, 1989; Bowen-Walker et al., 1999; Nordström, 2000). Using RT-PCR protocols, DWV has been detected in all honey bee life stages, as well as glandular secretions (Chen et al., 2005; Yue and Genersch, 2005). There are two main transmission routes. Oral transmission between bees is thought to propagate the inapparent, persistent infections whereas pupae infected through injection by the vector *V. destructor* sometimes develop into adult bees showing wing and other morphological deformities, a reduction in emergence size, or die during pupation (Bailey and Ball, 1991; Yue and Genersch, 2005). Serological tests as well as a quantitative RT-PCR analysis have shown significantly elevated DWV titres in deformed bees, compared to normal adults emerging from mite-infested cells, with lower titres in bees emerging from non-mite-infested cells (Ball, 1989; Bowen-Walker et al., 1999; Chen et al., 2005; Nordström, 2000). So far, DWV has been considered a pathogen with high host specificity (Allen and Ball, 1996). Recently, however, evidence was obtained suggesting that DWV may also replicate in *V. destructor* (Ongus et al., 2004; Yue and Genersch, 2005), perhaps indicating a broader host range for this virus.

In the year 2004, two independent observations were made of bumble bees exhibiting wing deformities. From March 2004 onwards, dead bumble bee queens (*Bombus terrestris*) with crumpled, vestigial wings (Fig. 1A) were found in European commercial bumble bee breeding operations at a frequency of around 10%. Frequently, young honey bees

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0022-2011/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jip.2005.10.002

are used in such operations to stimulate hibernated queens to initiate nesting behavior, thus providing a close interface between the two species. In the autumn of the same year in Germany, a feral bumble bee colony (*Bombus pascuorum*) living in close proximity to several honey bee hives (*Apis mellifera carnica*) showing clinical symptoms of infection with DWV were observed to contain workers with crumpled wings. Worker bumble bees from this colony had been observed to rob honey from the neighboring honey bee colony. No *V. destructor* mites could be found in this bumble bee colony. Since crumpled and vestigial wings in honey bees are considered a clinical symptom of DWV infection, we investigated whether these symptoms in bumble bees were associated with the presence of DWV.

Following sensitive and specific RT-PCR protocols for the detection of DWV in honey bees (Genersch, 2005; Yue and Genersch, 2005), total RNA extracted separately from head, thorax, and abdomen of crippled and asymptomatic bumble bees (RNeasy, Qiagen) was analyzed for the presence of DWV sequences. RNA extracted in the same way from crippled and phenotypically normal honey bees served as controls. A panel of 10 DWV-specific PCR primer pairs (Table 1) was used for RT-PCR analysis (Genersch, 2005). One-step RT-PCRs (Qiagen) were performed with incubations for 30 min at 50 °C, 15 min at 95 °C followed by 35 cycles with 30 s at 94 °C, 30 s at 54.3 °C, and 30 s at 72 °C, followed by a final elongation step for 10 min at 72 °C. Together, the 10 PCR target regions covered 45% of the 10,131 nt DWV genome. All bumble bees (*B. terrestris* and *B. pascuorum*) exhibiting deformed wings tested positive for DWV-RNA with all primer pairs in the thorax and abdomen but not in the head. DWV RNA could not be detected in asymptomatic bumble bees (Fig. 1B). In contrast, asymptomatic honey bees contained viral RNA in thorax and abdomen while deformed honey bees were positive for DWV sequences in all body parts (Fig. 1B).

Table 1
Position of primer pairs, length of amplicons, and nucleotide identity of sequences obtained with the Italian DWV sequence (Lanzi and Rossi, GenBank Accession No. AJ489744)

Primer pair	Amplicon location (AJ489744 numbering)	Amplicon length (bp)	% identity to AJ489744
DWV-R/DWV-S	1–195	195	99.5
F666/B1180	666–1180	515	99.6
F1135/B1585	1135–1585	451	99.6
F1/B1	3722–4076	355	100
F2/B3	4227–4794	568	100
F4/B5	4781–5296	516	100
F6/B8	5770–6162	393	99.5
F7/B11	6758–7236	479	98.3
F10/B16	7467–8062	596	99.5
F15/B23	9247–9697	451	100

The electrophoretic mobility of the RT-PCR amplicons correlated with their expected sizes. The specificity of the amplicons was further verified by directly sequencing the RT-PCR products (all F/B primer pair amplicons: Medigenomix, Martinsried, Germany; DWV-R/DWV-S amplicon: Nucleic Acid Facility, Penn State, USA). The sequences were aligned to the Italian DWV sequence (Lanzi and Rossi, GenBank Accession No. AJ489744) using CLUSTAL-W (Thompson et al., 1994). Amplicons generated with primer pairs F1/B1, F2/B3, F4/B5, and F15/B23 were identical to the Italian DWV sequence, whereas amplicons generated with primer pairs F6/B8, F7/B11, F10/B16, F666/B1180, and F1135/B1585 revealed 99.5, 98.3, 99.5, 99.6, and 99.6% identity, respectively (Table 1). The cloned amplicons generated with DWV-R/DWV-S were derived from three sources; deformed bumble bee queens, their corresponding bumble bee workers, and the attendant honey bees found in the same hive. The sequences from all these sources were identical to each other and 99.5% identical to the Italian DWV sequence. These three RNA samples tested negative by RT-PCR for the presence of Kashmir bee virus (KBV) and acute bee

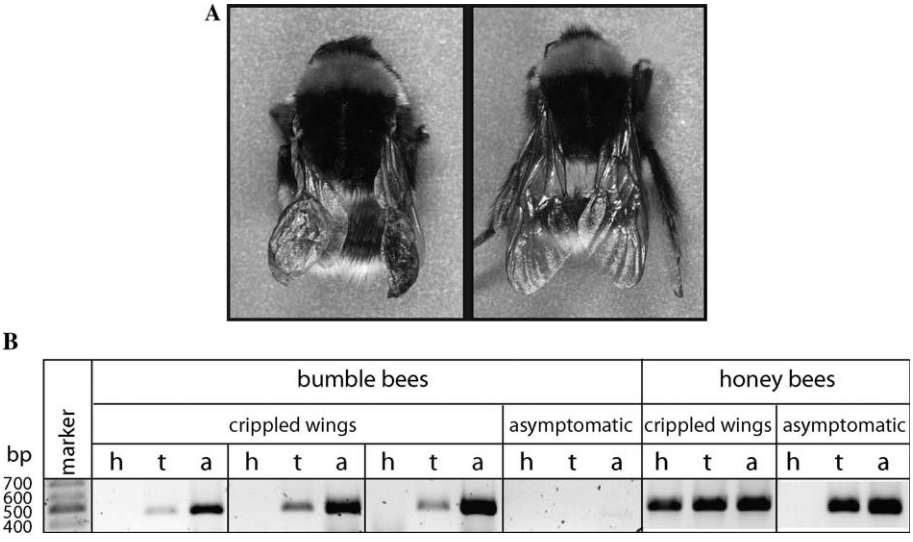

Fig. 1. Evidence for DWV infection in bumble bees. (A) Crippled (left) and healthy (right) bumble bees (*B. terrestris*). (B) RT-PCR analysis of DWV in head (h), thorax (t), and abdomen (a) of crippled and asymptomatic bumble bees (*B. pascuorum*) and honey bees (*A. mellifera*). Representative results obtained with primer pair F15/B23 are shown.

paralysis virus (ABPV), two other viruses commonly associated with varroa infestation in honey bees (Bailey and Ball, 1991; Ball, 1989; de Miranda et al., 2004). The sequences of all amplicons together were 99.6% identical to the Italian DWV sequence.

These results convincingly demonstrate that the bumble bees analyzed were infected by DWV. DWV infection in bumble bees correlated with wing deformities suggesting that DWV causes crippled wings not only in honey bees but also in bumble bees. Thus, either DWV has broader host specificity than anticipated or we are just observing DWV switching to another host and thereby broadening host specificity. The latter possibility is consistent with a potentially higher virulence of DWV in bumble bees as compared to honey bees as it is well known that pathogens alter virulence when they switch to a new host. Differences in virulence are suggested by the fact that asymptomatic honey bees did contain DWV in thorax and abdomen, while for bumble bees the same result correlated with deformed wings. Additionally, in honey bees oral transmission of DWV through feeding is not usually sufficient to cause crippled wings but transmission has to occur injection-like through *V. destructor* during pupal stages (Bailey and Ball, 1991). In contrast, DWV-infected bumble bees showed wing deformities in the absence of *V. destructor*. Since the oral route is the most likely route of transmission in this case, our results suggest that orally transmitted DWV can lead to wing deformity in bumble bees. Our results also reveal differences in tissue tropism between DWV-infected, clinically diseased honey bees and bumble bees. While crippled honey bees were characterized by virus detection in total RNA from head, crippled bumble bees tested negative for DWV in head RNA.

Although some observations could suggest a novel introduction of DWV from honey bees to bumble bees, DWV is not the only virus described from honey bees that has a broader host range. For example, Kashmir bee virus (KBV) has also been detected in the German wasp (*Vespa germanica*) (Anderson, 1991), acute bee paralysis virus (ABPV) is also present as inapparent infections in a range of *Bombus* spp., (Bailey and Gibbs, 1964), and many *Apis mellifera* viruses and other pathogens–parasites can also infect the other *Apis* species (Allen and Ball, 1996; Grabensteiner et al., 2001; Morse and Flottum, 1997). Whether DWV also occurs in bumble bee populations without close contact to infected honey bees is not presently known.

In conclusion, we demonstrated that DWV is pathogenic to at least two bumble bee species (*B. terrestris* and *B. pascuorum*) causing wing deformity similar to clinically DWV-infected honey bees. Since DWV infection of bumble bees occurred under natural conditions further studies are necessary to determine the incidence and prevalence of DWV in bumble bee populations.

Acknowledgments

E.G. and C.Y. were supported by the EU (according to regulation 1221/97) and by grants from the Ministries of Agriculture of Brandenburg, Sachsen, and Thüringen, Germany, and the Senate of Berlin, Germany. J.R. de M. was supported by grants from the Pennsylvania Department of Agriculture (ME441833) and the Binational Agriculture Research and Development Foundation (US-3205-01R).

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