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Detection of deformed wing virus in *Vespa crabro*

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Abstract

Specimens of *Vespa crabro* L. queen were found to be infected by deformed wing virus (DWV). The abdomen and the thorax of asymptomatic and symptomatic wasps were positive for the virus by strand specific RT-PCR, indicating active replication. This finding confirms the ability of the virus to infect not only bees (Apoidea) but also wasps (Vespoidea) suggesting a possible transmission route by ingestion of infected honey bees by wasp's larva. This is the first report concerning the detection of DWV in *V. crabro*. In the view of this finding the possibility of using naturally infected bees as a tool for the biological control of its predators is discussed.

Key words: deformed wing virus, honey bees, European hornet, strand specific RT-PCR.

Introduction

Deformed wing virus (DWV) is a pathogen of honey bees (*Apis mellifera* L.) and it is linked to colony bee losses causing important negative effects for the ecosystem, the agriculture and the economy (de Miranda and Genersch, 2010; Genersch and Aubert, 2010).

DWV belongs to the Picornaviridae family within the *Iflavirus* genus, with a positive ssRNA genome of 10 kb in length (Lanzi *et al*., 2006).

Recently, three genetic variants of DWV defined as type A, B and C have been identified (McMahon *et al*., 2016; Mordecai *et al*., 2016). By performing laboratory experiments and a systematic field survey DWV type B resulted as more virulent than the established DWV type A (McMahon *et al*., 2016).

DWV, with some exception, is distributed worldwide and is transmitted to honey bees mainly by the bite of the ectoparasitic mite *Varroa destructor* Anderson et Trueman which is its main biological vector. The role of varroa mite in transmitting DWV is relevant in permitting the infection of new bee families. Within a bee family/beehive transmission could also occur horizontally through direct contact between infected and non-infected bees, especially when the level of infection (infectious titers) is high (Ball and Allen, 1988; Nordström, 2003; Shen *et al*., 2005; Lanzi *et al*., 2006; Gisder *et al*., 2009; Martin *et al*., 2012; Francis *et al*., 2013; Giusti *et al*., 2016). DWV can also be transmitted vertically, and recent studies have proven the presence of DWV on flower pollen, pollen load and in other bee products supporting the horizontal transmission of the pathogen (Chen *et al*., 2006; Yue *et al*., 2007; Möckel *et al*., 2011; Mazzei *et al*., 2014). The virus can persist in the bee colony as covert asymptomatic infection, but as consequence of high level of virus particle production, overt infections are revealed (de Miranda and Genersch, 2010). High levels of viral infections are often triggered by change in the homeostasis of the bee-family such as in case of high levels of varroa mite infestation (Francis *et al*., 2013). Those are characterized by deformed or missing wings, shortened abdomens and premature death leading ultimately to the collapse of the bee colony (de Miranda and Genersch, 2010). Several chemical and natural approaches have been proposed for controlling DWV infection (Desai *et al*., 2012; Mazzei *et al*., 2016). Honey bees are not only exposed to pathogens; vertebrate and invertebrate predators are an important threat for the entire colony.

Among predators, some insects of the *Vespa* genus are extremely dangerous for the bee colony, even causing its complete collapse.

The European hornet (*Vespa crabro* L.) is one of the honey bee's natural predators and it is widely distributed in Italy. The hornet is a carnivore predator of other wasps, large moths and honey bees. It can build impressive nests and its dimensions can easily reach up to 2.5 cm or 5 cm in length for the adult male and female, respectively (Carpana and Lodesani, 2014). Honey bees can be attacked by *V. crabro* during their foraging flight or just outside the hive. In case the colony is already suffering for other pathologies, *V. crabro* could have a devastating effect on it (Baracchi *et al*., 2010). *V. crabro* is dangerous also for humans since its sting is very painful and, in some cases, could even be lethal (Antonicelli *et al*., 2003).

In this short report, we describe the first detection of DWV from *V. crabro* by molecular investigation. DWV was detected from two insects of which one was presenting deformed wings. Implication in using DWV as a biological control tool against wasps is here suggested as a possible perspective.

Materials and methods

Sampling

In October 2016, a *V. crabro* nest was destroyed at the Department of Veterinary Science, University of Pisa, Italy (43°70'85"N 10°41'07"E). Two new generation queens were caught alive and one of them was showing deformed wings (figure 1). Insects were immediately stored at −80 °C. The rest of the insects could not have been tested since destruction was performed by flaming the nest.

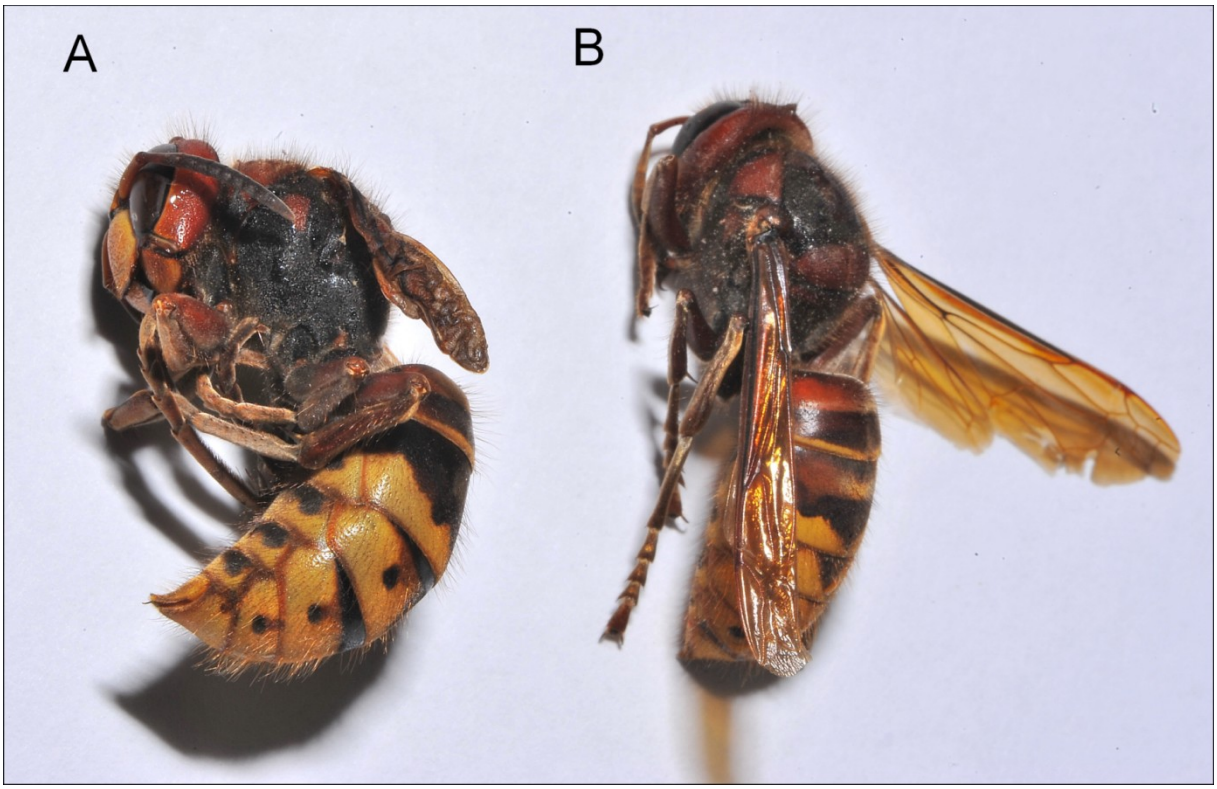

Figure 1. *V. crabro* queens: **A)** Symptomatic wasp presenting short and crippled wings; **B)** Asymptomatic wasp.

Total RNA extraction

Head, thorax-abdomen sections of each wasp were divided longitudinally in two parts and analyzed separately. Each part was homogenized using a Tissue Lyser II (Qiagen, Hilden, Germany) for 3 minutes at 25 Mhz. Total RNA was extracted with RNeasy Kit following manufacture instructions (Qiagen). Samples were eluted in 30 µl RNase-free water and stored in aliquots at −80 °C. As a negative control, a flesh fly (Sarcophagidae) collected in the same area was dissected and analyzed following the same protocol.

Strand-specific RT-PCR

A two-step RT-PCR was used for the specific detection of positive and negative strand DWV RNA. Reactions were set up as previously described (Mazzei *et al*., 2014). Five microliters of the obtained cDNAs were used as template for the PCR reaction. The reaction was carried out with HotStarTaqPlus Polymerase Mix (Qiagen), in presence of primers Fw 8450 5'-TGGCATGCCTT GTTCACCGT-3' (nt. 8450-8469) or Rev 8953 5'-CGTGCAGCTCGATAGGATGCCA-3' (nt. 8953-8932), which amplify a 504 bp fragment relative to the highly conserved region coding for RNA-dependent RNA polymerase (Rd-Rp). The nucleotide positions cited throughout the text refer to the DWV reference sequence (NC_004830.2).

Sample sequencing and phylogenetic analysis

PCR positive samples were chosen for sequence analysis by using primers Fwd 8450 and Rev 8953 (BMR, Padova). Results were analyzed by BioEdit software (Hall, 1999). Molecular phylogenetic analysis of a set of DWV sequences was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. A total of 499 nucleotides per sequence were included in the final dataset.

Results

Detection of replicating virus in *V. crabro*

DWV is a positive-strand virus, therefore negative-strand RNA is only found when the virus is replicating. A strand-specific RT-PCR assay demonstrated the presence of replicating virus (- ssRNA) in the abdomen and thorax of asymptomatic and symptomatic *V. crabro* (figure 2A). On the contrary, we did not find either replicating or genomic RNA in the heads of the analyzed specimens.

Sequence analysis

In order to confirm the PCR results, DNA positive samples were sequenced. Aligning of the partial sequence of our asymptomatic and symptomatic wasps with the consensus sequence of DWV-A, DWV-B and DWV-C types (accession numbers NC_004830.2, NC_006494.1 and CEND01000001 respectively) indicate that samples show high similarity to the less virulent DWV- A type (figure 2B). This data was further supported by phylogenetic analysis performed to compare our DWV sequence isolated from *V. crabro* (504 bp) to other DWV sequences from *A. mellifera* available on GenBank and indicate a close similarity with the sequence obtained from infected honey bees collected in the area nearby the *V. crabro* nest (figure 3).

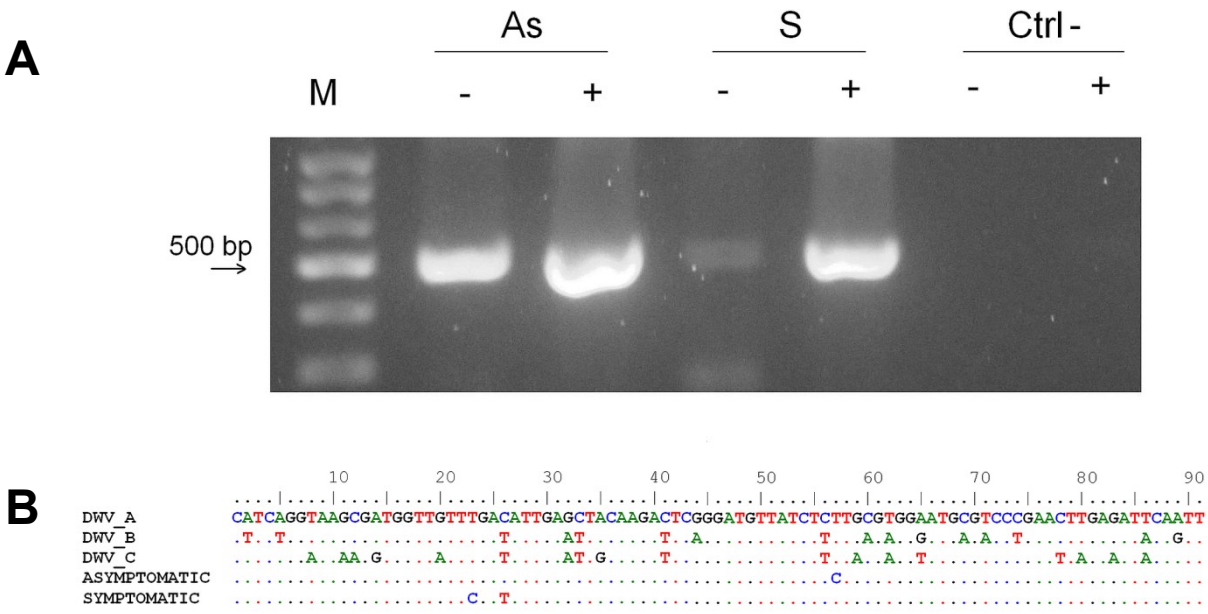

Figure 2. Evidence of replicating and genomic DWV in *V. crabro*: **A)** Gel electrophoresis of strand specific RT-PCR performed on cDNA obtained from thorax and abdomen of Asymptomatic (AS) and Symptomatic (S) wasps. Replicative strand (-) and genomic strand (+). As a negative control cDNA obtained from a flesh fly (Sarcophagidae) was run in parallel; **B)** Sequence alignment of *V. crabro* DWV amplicons with reference, DWV-A, DWV-B and DWV-C types (accession numbers NC_004830.2, NC_006494.1 and CEND01000001 respectively).

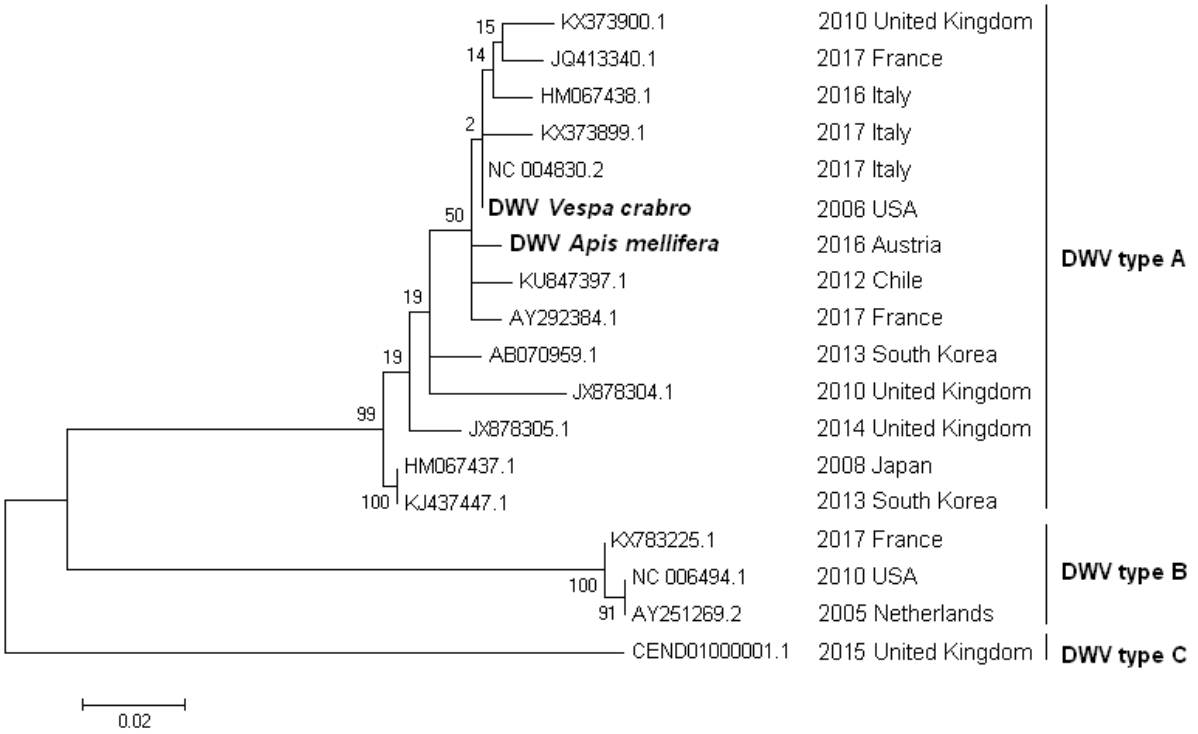

Figure 3. Molecular Phylogenetic analysis by Maximum Likelihood method. The percentage of trees in which the associated taxa clustered together is shown next to the branches DWV sequences accession numbers are reported and associated to year and site of origin. Bold characters indicate DWV sequence obtained in this study.

Discussion

Several studies have shown that pollinator insects can be infected by DWV (Genersch *et al*., 2006; Singh *et al*., 2010; Rader *et al*., 2015; Gisder and Genersh, 2016; Tehel *et al*., 2016). Interestingly, DWV has been recently detected in the invasive Argentine ant [*Linepithema humile* (Mayr)] suggesting its possible role as reservoir for the honey bee pathogen (Sébastien *et al*., 2015; Gruber *at al*., 2017).

Rader *et al*. (2015) demonstrated that *Vespula vulgaris* L. could be infected by DWV without showing any DWV symptoms. In *Vespula* spp. and in this latter *V. crabro* case, DWV has shown its ability to jump from one superfamily (Apoidea) to another (Vespoidea). Being an RNA virus could provide to DWV an increased adaptation ability. RNA viruses, compared to DNA viruses, have a higher mutation rate, which is mainly due to the lack of proofreading activity of their RNA depended-RNA polymerases. This error prone replication may be very important in virus evolution since it may result in an increased ability to adapt to a new host (Ferrer-Orta *et al*., 2015; Morley *et al*., 2015). We are not in the position to explain when and how the European hornets were infected with DWV. As *V. crabro* is neither a pollinator or susceptible to *V. destructor* we can exclude transmission by exposition to flower pollen and parasite bite. So far, we can formulate the hypothesis that wasps could have been exposed to the virus by eating DWV infected honey bees during their larval state. To support such hypothesis, we should remark that an apiary previously tested as infected by DWV was present in the close neighbourhood of the *V. crabro* nest. The presence of genomic and replicating RNA in the abdomen and thorax of *V. crabro* specimens demonstrate that, whatever was the route of infection, DWV is able to adapt and replicate to this new host. The absence of virus within the heads agree with results reported for bumblebees by Genersch *et al*. (2006). By comparison of sequences detected in *V. crabro* with those belonging to the established DWV-A, B and C types (McMahon *et al*., 2016; Mordecai *et al*., 2016), we can state that DWV detected in *V. crabro* shows high similarity to the less virulent DWV-A type. This result is not surprising since honey bees collected all year round in the area surrounding the sampled nest are also linked to DWV-A (Forzan *et al*., 2017).

The ability of DWV to jump between superfamily taxa showed by DWV opens a new epidemiological scenario concerning relationship among ecosystems that need to be further investigated.

The presence of replicating DWV with overt infection in *V. crabro* leads to hypothesize the development of a possible system to protect healthy honey bee's colonies from *Vespa velutina* Lepeletier, a dangerous invasive alien honey bee's predator recently introduced in Europe from South East Asia (Monceau *et al*., 2014). This system could be based on the use of naturally highly infected bee colonies as possible prey.

Additional studies need to be performed in this direction, since to our knowledge it is not clear if infection of DWV in *V. crabro* or other predators could have lethal consequences. The pathological aspect of DWV infection in *V. crabro* could be confirmed by performing experimental infection in wasp's larvae (*V. crabro* and *V. velutina*).

In conclusion, in this paper we provide evidence of the first detection of replication competent DWV from symptomatic and asymptomatic European hornet queens (*V. crabro)*.

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