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Michael Eyer, Yan Ping Chen, Marc Oliver Schäfer, Jeff Pettis, Peter Neumann. Small hive beetle, Aethina tumida, as a potential biological vector of honeybee viruses*. Apidologie, Springer Verlag (Germany), 2009, 40 (4), <10.1051/apido:2008051>. <hal-00891994>

HAL Id: hal-00891994

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Apidologie 40 (2009) 419–428
© INRA/DIB-AGIB/EDP Sciences, 2009
DOI: 10.1051/apido:2008051

Available online at:
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Original article

Small hive beetle, *Aethina tumida*, as a potential biological vector of honeybee viruses*

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Received 17 June 2008 – Revised 14 August 2008 – Accepted 25 August 2008

Abstract – The small hive beetle (SHB, *Aethina tumida*) is a parasite and scavenger of honeybee colonies. Here, we conducted laboratory experiments to investigate the potential of SHB as a vector of honeybee viruses. Using RT-PCR methods, Deformed Wing Virus (DWV) was detected in adult SHBs that: (1) were fed with dead workers with deformed wings, (2) were fed with DWV-positive brood, and (3) were associated with DWV-contaminated wax. SHB became significantly more often infected through feeding on virus infected workers, brood and the virus contaminated wax compared to pollen and the controls, where no infections were found. DWV was also detected in adult SHB after trophallaxis with infected workers. Further, among SHBs identified as DWV-positive, 40% of beetles carried negative stranded RNA of DWV, indicating virus replication. Our results suggest that SHB can be infected with honeybee viruses via food-borne transmission and have the potential of being a biological vector of honeybee viruses.

***Apis mellifera* / *Aethina tumida* / biological vector / deformed wing virus / honeybees / small hive beetle**

1. INTRODUCTION

Recently, severe colony losses affected beekeepers all over the world (Cox-Foster et al., 2007). It is supposed that the interaction of multifactorial diseases may play an important role in these losses, since honeybees are threatened by numerous pathogens (Bailey and Ball, 1991). Interactions between honeybee viruses and other pathogens may be one of the mechanisms. Among honeybee pathogens, viruses pose one of the key threats to the health and well-being of their hosts (Chen and Siede, 2007). To date, at least 18 viruses have been reported to infect honeybees worldwide (Allen and Ball, 1996). Most of the honeybee viruses, reported so far, consist of a genome of positive–sense single-stranded RNA (Chen and Siede, 2007). The combination of viruses with the ectoparasitic mite, *Varroa destructor,* seems to be important in honeybee pathology (Martin, 2001; Bakonyi et al., 2002; Tentcheva et al., 2004a, b; Yue and Genersch, 2005). In fact, Bowen-Walker et al. (1999) demonstrated experimentally the transmission of DWV through this mite.

The small hive beetle (= SHB), *Aethina tumida* (Coleoptera: Nitidulidae) is a parasite of honeybee colonies native to sub-Saharan Africa (Lundie, 1940; Schmolke, 1974; Neumann and Elzen, 2004). It has become an invasive species (Neumann and

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Elzen, 2004; Neumann and Ellis, 2008) and a severe pest in some regions (USA: Elzen et al., 2000; Australia: Spiewok et al., 2007). Free-flying SHB adults invade host colonies over several kilometers, mate and reproduce within hives (Neumann and Elzen, 2004; Spiewok et al., 2008). Although the pest status of the SHB in honeybee colonies is now well known and control and diagnosis are available (cf. Neumann and Ellis, 2008; Schäfer et al., 2008), the role of the SHB as a vector of honeybee viruses has been neglected. There exist differential potential contamination pathways for oral uptake of honey bee viruses by SHB. For example, the beetles can exploit the trophallaxis feeding within the host colonies (Ellis et al., 2002b). As a defense strategy against the SHB, honeybees construct cells of propolis into which they drive the beetles and imprison those (Neumann et al., 2001a). Through behavioral mimicry, SHB can induce trophallaxis feeding from the honeybees (Ellis et al., 2002a), which is the only way for SHB to obtain food in such prisons except in rare cases of cannibalism (Neumann et al., 2001a). Another possible contamination route is through feeding on virus infected dead workers and bee brood, because small hive beetles may be also scavengers of honeybees. Moreover, the beetles also eat pollen (Ellis et al., 2002a), wax and other food resources (Buchholz et al., 2008) which can be contaminated with viruses. This could lead to further possible viral contamination.

To test for potential interactions between honeybee viruses and the SHB we used the Deformed Wing Virus (DWV) as an experimental virus model system. DWV is a very common honeybee virus (Chen and Siede, 2007) and the complete genome sequence has recently been determined (Lanzi et al., 2006). A routine survey, carried out in Beltsville, MD showed that DWV infection was present in 100% of the investigated apiaries (Chen and Siede, 2007). Typical symptoms of DWV infection include shrunken and crumpled wings, shortened body size and discoloration in adult bees (Chen and Siede, 2007). Bailey and Ball (1991) linked these symptoms with DWV infection by applying bio assay tests using bee pupae. The viruses often persist in bees in a latent state with horizontal and vertical transmission and pathogen-host interactions determining the fitness of the pathogens (Chen and Siede, 2007).

However, once ingested, viruses may replicate in the SHB, similarly as in *V. destructor* (Ongus et al., 2004; Yue and Genersch, 2005). If replication occurs, the negative-stranded DWV intermediates, indicative of the virus replication would be present in SHBs during the replication process. Considering the pedigree of the three species, this scenario seems plausible since SHBs (Insecta: Coleoptera: Nitidulidae) and bees (Insecta: Hymenoptera: Apidae) are both insects and therefore taxonomically more closely related than *V. destructor* (Arachnida: Acari: Varroidae) and bees. Based on the above discussed issues, we developed the following hypotheses:

Hypothesis 1: Adult SHB become infected with DWV through feeding on dead adult workers with clinical symptoms.

Hypothesis 2: Adult SHB become DWV infected through feeding on infected honeybee brood.

Hypothesis 3: Adult SHB become infected with DWV via feeding on virus-contaminated pollen.

Hypothesis 4: Adult SHB become infected via association with contaminated wax.

Hypothesis 5: Negative-stranded RNA of DWV can be detected in the SHB indicating virus replication.

Here, we test these hypotheses using classical SHB cage experiments (Elzen et al., 2001) and RT-PCR assays.

2. MATERIALS AND METHODS

Experiments were conducted at the USDA-ARS Bee Research Laboratory in Beltsville (Maryland, USA) from September–December 2007. Honeybee colonies (N = 10) of mixed European origin, predominantly *A. m. ligustica*, were not treated against the ectoparasitic mite *V. destructor* to facilitate DWV infection (Chen and Siede, 2007). Adult SHB were collected from locally infected colonies (N = 20) and used to initiate a laboratory rearing following standard protocols (Neumann et al., 2001b; Mürrle and Neumann, 2004). For each

experiment, the respective working hypotheses are given in italics below.

2.1. Feeding experiments

Experiment 1: workers with deformed wings

Hypothesis: Adult SHB become infected with DWV via feeding on dead adult workers with deformed wings

Freshly emerged workers with deformed wings, a clinical symptom of DWV infection (Chen and Siede, 2007), were collected from the brood nests and bottom boards of 10 colonies (see above) and immediately stored at −80 °C. Plastic containers [6.8 × 7.5 × 9.7 cm] were each equipped with an 8-mL-capacity vial filled with water and SHB oviposition sites (two double microscope slides spaced by cover slips; Hoffmann et al., 2008). SHB oviposition sites were added to enhance the likelihood of scavenging by female SHB to meet their protein supply. Ten dead workers with deformed wings were put into each treatment container (N = 9). To further facilitate SHB scavenging, the abdomens of these workers were slightly cut with a scalpel. The control containers (N = 3) were supplied with sugar water only (8 mL, in a ratio of 1 sugar/3 water). Then, 12 adult SHB from the laboratory rearing (24–48 hours old, unfed) were transferred into each container and kept for six days in an incubator at 30 °C and 60% RH. All collected SHBs were stored immediately at −80 °C until RNA extraction. Four SHBs from each cage and ten workers showing clinical symptoms were analyzed individually for the presence of DWV.

Experiment 2: DWV positive brood

Hypothesis: Adult SHB become DWV-infected via feeding on honeybee brood

A piece of brood comb [7 × 10 cm] with open and sealed brood from a colony infected by DWV was placed into a plastic box [14 × 19 cm; hole with mesh wire on top 8 × 13 cm]. Twenty adult SHB (24–48 hours old, unfed) were removed from the laboratory rearing and introduced into the box and kept for seven days in an incubator at 30 °C and 60% RH. After incubation, all SHB were stored immediately at −80 °C for subsequent RNA extraction and molecular analysis. Additionally, 20 adult SHB were collected from the laboratory rearing and subjected to examination for DWV directly as a negative control of the experiment.

Experiment 3: DWV-positive pollen

Hypothesis: Adult SHB become infected with DWV via feeding on virus-contaminated pollen

An identical set up with the same conditions as in Experiment 1 was used, except the boxes (N = 3) were supplied with 25 g of pollen that was previously confirmed as DWV-positive and eight adult SHBs were introduced into each box. After incubation, four SHBs from each cage were randomly sampled and analyzed individually for DWV infection.

Experiment 4: DWV-contaminated wax

Hypothesis: Adult SHB become infected via association with contaminated wax

Following the same set up and conditions as in Experiment 1, another treatment was performed with DWV-contaminated wax. The boxes (N = 6) were each provided with 4 × 3 cm wax-slides and 12 adult SHB. Again, four beetles of each cage were randomly collected and analyzed for DWV infection.

2.2. Trophallaxis experiment

Hypothesis: Adult SHB become DWV-infected via trophallaxis with infected workers

Twelve experimental cages [9 × 8 cm] with wire screens were partitioned between the upper 5 cm (for bees) and the lower 3 cm (for SHB) by metal gauze that prevented mingling of workers and SHB, but did allow antennal and mouthpart contact, hence trophallaxis, between bees and SHB through the gauze mesh (Ellis et al., 2002b). A brood frame with sealed worker brood from a DWV-infected colony was put into an incubator. After 24 hours, 25 newly emerged workers were put into the upper parts of each of the six treatment cages. The workers were supplied with a honey-pollen paste (ratio: honey/pollen; 3/1) and water filled in a 50- mL vial. For the control cages (N = 6), 3 cm high compartments were supplied with sugar water (8 mL vials). To facilitate trophallactic contacts, the lower

SHB compartments in the treatment cages (N = 6) were only supplied with water. Then, 12 adult SHBs from the laboratory rearing (24–48 hours old, unfed) were transferred into the lower compartment of each cage and kept for eight days in an incubator at 30 °C and 60% RH. After the experiment, all SHB were stored immediately at −80 °C until RNA extraction. Four SHB and five workers from each cage were analyzed individually for DWV infection status.

2.3. RNA analyses

2.3.1. Extraction

Total RNA was extracted from SHBs, bees, wax and pollen that were collected during feeding and trophallaxis experiments using TRIzol, a reagent for the isolation of total RNA (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. In brief, samples of the SHBs and bees that were stored at –80 °C were individually homogenized in TRIzol and chloroform to remove proteins, lipid, and DNA. After precipitation of RNA with isopropanol, the RNA pellet was washed with 70% ethanol. The resulting RNA pellet was resuspended in DEPC-treated water and a ribonuclease inhibitor (RNaseOut Ribonuclease, Invitrogen, Carlsbad, CA) was added. The concentration of RNA was checked by spectrophotometric measurement at 260 nm.

2.3.2. RT-PCR Amplification

The DWV-specific primers used in this study were DWV-sense (5'-ATCAGCGCTTAGTGGAGGAA-3'), and DWV-antisense (5'-TCGACAATTTTCGGACATCA-3', Chen et al., 2005). The primer pair was expected to generate a 702-bp PCR product. Access RT-PCR system (Promega, Madison, WI) was used for RT-PCR reaction according to the manufacturer's instructions. The reaction was performed in a total volume of 25 μL with a final concentration of 1X of AMV/Tfl reaction buffer, 0.2 mM of dNTP, 1 μM of sense primer, 1 μM of antisense primer, 2 mM of $MgSO_4$, 0.1 unit of AMV reverse transcriptase, 0.1 unit of T*fl* DNA polymerase and 500 ng of total RNA. Amplification was performed using the PTC-200 DNA Engine (MJ Research, Waltham, MA) with the following thermal cycling profiles: one cycle at 48 °C for 45 min for reverse transcription; one cycle at 95° for 2 min; 40 cycles consisting of 95 °C for 30 s, 55 °C for 1 min, and 68° for 2 min; one cycle at 68 °C for 7 min. Negative (water) and positive controls (previously identified positive) were included in each run of the RT-PCR reaction to detect any form of contamination and to verify if RT-PCR failure was due to RNA degradation, respectively. PCR products were electrophoresed in 1% agarose gels containing 0.5 μg/mL ethidium bromide and visualized under UV light to check for the expected size fragments.

2.3.3. Detection of the negative-stranded DWV

Hypothesis: Negative-stranded DWV RNA can be detected in SHB indicating virus replication

In order to prove whether the SBH could serve as a biological vector, we investigated the presence of negative-stranded DWV RNA intermediates, indicative of virus replication, in the SHBs using strand-specific RT-PCR, following the method developed by Yue and Genersch (2005). First-strand cDNA was synthesized from total RNA extracted from experimental SHBs with Tag-DWV-antisense primer (5'-agcctgcgcacgtggTCGACAATTTTCGGACATCA-3') using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. The sequence of Tag is shown in lowercase and was published by Yue and Genersch (2005). After first-strand cDNA synthesis, amplification of cDNA was conducted with a Tag and DWV-sense primer set. Polymerase chain reaction (PCR) was performed in a 50 μL reaction mixture containing 0.2 mM of dNTPs, 1.5 mM of $MgCl_2$, 0.2 μM of each primer, 2.5 units of Taq DNA polymerase (Invitrogen, Carlsbad, CA), and 2 μL of cDNA from the first-strand reaction. PCR was performed in the following thermal profile: one cycle of 95 °C for 2 min, followed by 30 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min, and a final step at 72 °C for 10 min. In every run of the amplification, a negative control without template DNA was included. A positive control was not included to avoid potential contaminations. The amplified products were electrophoresed on a 1.5% low-melting-point agarose gel and visualized under UV light as described above.

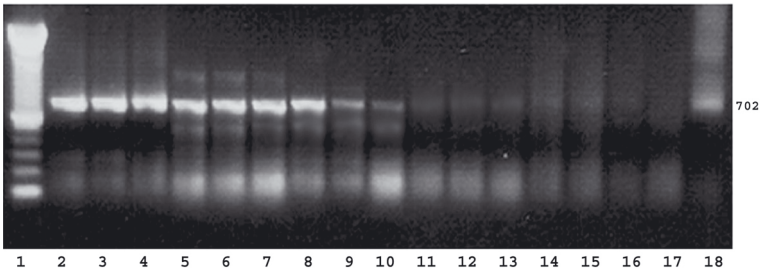

Figure 1. Resources that SHB acquired DWV. A 702-bp long band indicating DWV-infection was found in SHB that were kept on dead workers with clinical symptoms (lanes 2-4), on DWV positive brood (lanes 5-7), on contaminated wax (lanes 8-10) and in the positive control (lane 18). No band was obtained for the SHB that were kept on pollen (lanes 11-13), on sugar water (= controls; lanes 14-16) and in the negative control (lane 17; lane 1: 100-bp DNA ladder).

2.3.4. Sequencing analyses

To confirm the specificity of the PCR amplification, DWV-specific fragments amplified from both positive and negative strands of viral RNA were purified using the Wizard PCR Prep DNA Purification System (Promega, Madison, WI) and sequenced from both forward and reverse directions. The nucleotide sequences of PCR products were analyzed and compared with sequences published at the GenBank, National Center for Biotechnology Information, NIH (http://www.ncbi.nlm.nih.gov/) using Blast.

2.4. Statistical analyses

A Kruskal-Wallis test was applied to compare the different groups of the feeding experiments. A Kolmogorov-Smirnov test was then used to see if there are any differences between these groups. All statistical analyses were performed using the program Statistica©.

3. RESULTS

3.1. Feeding experiments

The RT-PCR assay showed that a 702-bp long band indicating DWV infection was found in SHBs that were fed with dead adult workers with deformed wings, DWV-infected brood, and DWV-contaminated wax (Fig. 1). No DWV specific signal was detected in SHBs that were supplied with pollen and sugar water (Negative control; Fig. 1).

In the treatment group fed with adult worker bees with deformed wing, DWV was detected in 97% of the SHB (N = 36). However, no DWV was found in any SHB from the control group (N = 12). The workers with deformed wings showed an 80% DWV infection rate (N = 10). Casual observations showed that almost all SHB eggs were found on the dead bees with only few on the oviposition sites.

In the treatment group fed with DWV-infected brood, all tested SHB were DWV positive (N = 12). No SHB directly collected from the rearing programm showed any DWV infection (N = 20).

In the treatment group supplied with DWV-positive pollen, all analyzed SHBs (N = 12) were DWV negative.

In the treatment group kept on DWV-contaminated wax, 91% of the beetles were DWV positive (N = 24).

The Kruskal-Wallis ANOVA shows that there is a significant difference between the treatments and the controls (DF = 4; Kruskal-Wallis test statistic; H = 77.171; $P < 0.0001$). The Kolmogorov-Smirnov post hoc tests revealed that the treatments with workers, brood and wax are significantly different from both treatment with pollen and sugar water (negative control) at $P < 0.01$, but not from each other at $P > 0.05$. Similarly, there was no significant difference between the pollen treatment and the control ($P > 0.05$).

3.2. Trophallaxis experiment

The analyses revealed that 41% of the 24 tested adult SHB were DWV positive. In contrast, all of the 24 beetles from the control group were DWV negative. Furthermore, 70% of the tested workers (N = 25) showed DVW presence.

3.3. Negative-stranded DWV

Among SHB which were fed on infected workers and previously tested positive for DWV, 40% showed the presence of negative-stranded DWV RNA (Fig. 2).

4. DISCUSSION

Our data provide the first evidence of the presence of honeybee viruses in SHB. Moreover, the results suggest that the SHB can become infected with DWV: (1) through feeding on workers with deformed wings, (2) through feeding on DWV-positive brood, and (3) in association with contaminated wax. In contrast, no evidence was found for any infection through DWV-contaminated pollen. However, trophallaxis with infected workers also resulted in DWV transmission to SHBs. Additionally, the negative stranded RNA of DWV was also detected in SHB, indicating that this beetle constitutes an alternative host for DWV replication and has the potential to serve as a biological vector for DWV within and between honeybee colonies.

Although all apiaries screened during the routine survey in Beltsville were positive for DWV (Chen and Siede, 2007), not all workers necessarily show virus infections. Indeed, when comparing pooled samples and individual bees from infected honeybee colonies, only a small proportion of bees without clinical symptoms showed DWV infections while the majority was uninfected (Berthoud and Neumann, unpublished data). The same is probably true for SHB in those colonies. Moreover, DWV infections of colonies show a seasonal fluctuation with higher infection levels in autumn (Tentcheva et al., 2004a, b), probably due to *V. destructor* being a vector (Chen and Siede, 2007). Indeed, the local survey for DWV in Beltsville was conducted in autumn (Chen and Siede, 2007). Finally, few adult SHB (N = 20) were used to initiate the laboratory rearing program, thereby further limiting chances of obtaining previously infected SHB. Therefore, it is not surprising that the SHB collected during summer to initiate the laboratory rearing did not show any DWV infections. Since, we only report of results obtained from laboratory experiments, we can obviously not judge the rate of infections under natural conditions. However, the occasional incidence of adult SHB with deformed wings within honeybee colonies (J.S. Pettis and P. Neumann, personal observations), which is a clinical symptom of DWV infection in bees (Chen and Siede, 2007), suggests that natural infections occur in the field.

The detection of viruses in the hypopharnygeal gland of honeybees (Bailey, 1969), in the gut tissue of queens and their feces (Chen et al., 2006a, b; Ribière et al., 2007), as well as in the food resources and similar in all stages of bees (for SBV and KBV, Shen et al., 2005; Chen et al., 2006a) indicates the existence of food borne transmission pathway in honeybee colonies. However, the virus transmission via colony food has not been demonstrated experimentally. Here, we provide the first experimental evidence which includes hive products such as pollen and wax with regard to food-borne transmission of honeybee viruses to SHB. The significantly higher infection rates of adult SHB when feeding on workers with wing deformities or infected bee brood, and in association with virus contaminated wax suggest that these are more efficient ways for oral infection. This is the first experimental proof that honeybee viruses can be transmitted via association with bee wax. Since bees are often in contact with wax, the principle material used in nest construction, this contamination pathway may also be possible for honeybees. In light of the potential role of viruses for Colony Collapse Disorder (= CCD; Cox-Foster et al., 2007), the observed infection via wax and food might be a consideration by apiculturists facing major losses.

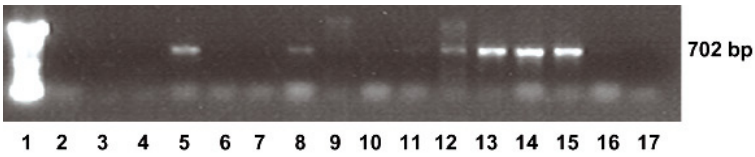

Figure 2. Detection of negative stranded RNA of DWV. Negative stranded RNA of DWV was detected in six (lanes 5, 8, 12-15) out of 15 SHB that were fed on infected honeybee workers and previously identified as being DWV positive by RT-PCR assay (lane 1: 100-bp DNA ladder; lane 17: negative control). No positive control was included in the assay to exclude the possible chance of contamination. The specificity of individual PCR bands was confirmed by sequencing.

Previous studies showed that bee viruses including Acute Bee Paralysis Virus (ABPV), Chronis Bee Paralysis Virus (CBPV), Kashmir Bee Virus (KBV), Sacbrood Virus (SBV) and DWV were detected in pollen samples in the colonies (Bailey, Bailey76; Shen et al., 2005; Chen et al., 2006a). However, no experiment was conducted to demonstrate that virus infections actually occur via feeding on such contaminated pollen. A study conducted by Chen et al. (2006b) showed that ABPV, CBPV, KBV, and SBV were detected in pollen samples but the same viruses were not detected in royal jelly, the glandular secretion of workers. This is similar to the present observation that no evidence of DWV infection in the SHBs through feeding on pollen was found. One explanation is based on the general detection of complex RNases (e.g. in *Arabidopsis thaliana*, Yen and Green, 1991) and RNA silencing processes in plants (Voinnet, 2001). It may be possible that pollen collected by the bees contained RNases, or some other antiviral substances which may have prevented spread of virus infections in bees and beetles. Similarly, an assumption was made by Morse (1972) that bees moisten the pollen with antibacterial components. So far, no studies are available that show antiviral components, which may be added by workers in stored pollen. In any case, it appears as if chances of transmission of DWV via contaminated pollen are lower compared to the other tested feeding substrates.

Further, SHB could become infected with DWV via exploitation of trophallaxis. This finding is in accordance with the detection of viruses in glandular secretions (SBV and KBV: Shen et al., 2005; DWV: Yue and Genersch, 2005) as well as in the hypopharnygeal glands of honeybees (SBV: Bailey, 1969), suggesting that SBV may be transmitted from nurse bees to larvae by feeding them. The observation of trophallaxis-virus-transmission to SHB implies that viruses can quite often be orally transmitted in honeybee colonies.

So far, only the role of the parasitic mite, *V. destructor*, has been documented as a biological vector of DWV (Bowen-Walker et al., 1999; Martin, 2001; Bakonyi et al., 2002; Tentcheva et al., 2004a, b; Yue and Genersch, 2005). Our detection of the DWV in SHB, suggest that these beetles are potential hosts for DWV and may also be involved in virus transmission among honeybees. This possible transmission pathway through the SHB would open a new route for the spread of honeybee viruses. In contrast to *V. destructor*, SHB are active flyers and have been reported to fly up to 16 km (cf. Neumann and Elzen, 2004, Spiewok et al., 2008). This could considerably favor the spread of viruses independent of the bees themselves, since the transmission process determines the spread of viruses in a population (Chen and Siede, 2007).

The detection of the negative-stranded RNA of DWV in SHB which had fed on infected workers indicates that the virus is able to replicate in SHB, similarly as in *V. destructor* (Ongus et al., 2004; Yue and Genersch, 2005). Clearly, there is the possibility that both positive and minus stranded DWV RNA might have been obtained by SHB while feeding on honeybee tissue. However, the high numbers of detected equivalent DWV copies in SHB (data not shown) are within the reported range

found for European *A. mellifera* bees and *V. destructor* (Tentcheva et al., 2006) despite the obvious size differences between SHB and bees. It is impossible that SHB completely obtain all virus copies while feeding on bees. Moreover, ongoing virus replication in the ingested honeybee tissue is most unlikely within the gut of SHB due to the activity of digestion enzymes rapidly destroying cell walls. Therefore, the high amounts of plus stranded RNA in combination with the detection of minus stranded DWV RNA in SHB suggest that this virus has not simply been stored but has instead infected SHB tissue and subsequently replicated.

This makes the SHB a potential biological vector of honeybee viruses. However, we found the DWV minus RNA in only 40% of the analyzed SHB samples. This is similar to earlier reports that DWV-replication is not found in all infected *V. destructor* mites (Yue and Genersch, 2005). That DWV replication was not found in all SHB could be due to an arrested replication mechanism through the host immune defense so that the virus remains in a latent stage in the host over time (Chen and Siede, 2007). Another possible explanation is that the virus is not able to replicate because suitable mutation/recombination, generating mutant quasi species (Holland et al., 1982; Domingo and Holland, 1997; Domingo et al., 1998; Vignuzzi et al., 2006), did not occur in all viruses.

The observation that *V. destructor* is a biological vector of DWV (Yue and Genersch, 2005) underlines the potential of DWV to persist in the environment as generalists. This is in agreement with the naturally high mutation rates of RNA viruses (Holland et al., 1982) and the predication of Woolhouse et al. (2001) that the consequent genetic variants should be more likely generalists. Taken together with the finding of DWV in bumble bees (Genersch et al., 2006) and in the ectoparasitic mite *Tropilaelaps mercedesae* (Dainat et al., 2008), the detection of CBPV in the ants *Camponotus vagus* and *Formica rufa* (Celle et al., 2008) and of KBV in the wasp *Vespula germanica* (Anderson, 1991), the detection of DWV in SHBs suggest strongly that the honeybee viruses can expand to multiple hosts. This indicates that the amount and role of shared pathogens has been considerably underestimated in the social insects and their associated arthropods, which could be relevant given the potential effects of host shifts for virulence (Woolhouse et al., 2001). Indeed, a potentially higher DWV virulence in bumble bees compared to honeybees has been reported (Genersch et al., 2006). This interaction between honeybee pathogens might be one mechanism contributing to the recent major losses of honeybee colonies. SHB may also play a role as a new host as well as a vector of honeybee viruses assisting their spread. However, the possible role of SHB as a vector of honeybee viruses and their impact on honeybee health require further studies.

In conclusion, our data show that SHBs can become infected with DWV through oral contamination pathways; via feeding on honeybee workers with deformed wings, by feeding on DWV-positive bee brood, in association with viral contaminated wax and by exploiting trophallaxis feeding. The detection of the minus RNA strand of DWV indicates virus replication. Finally, we conclude from our study that the SHB is a potential biological vector of honeybee viruses and it appears this could be relevant for honeybee pathology.

ACKNOWLEDGEMENTS

Appreciation is addressed to Michele Hamilton and Nathan Rice for assistance in the field and laboratory. Financial support was provided through the Swiss Academy of Sciences to M.E.

Le Petit coléoptère des ruches, *Aethina tumida*, vecteur potentiel des virus de l'Abeille domestique.

***Apis mellifera* / *Aethina tumida* / vecteur biologique / virus de l'aile déformée / DWV**

Zusammenfassung – Der Kleine Beutenkäfer, *Aethina tumida*, ist ein potentieller Vektor von Viren der Honigbienen. Der Kleine Beutenkäfer, *Aethina tumida* (Coleoptera: Nitidulidae), ist ein Parasit von Völkern der Honigbiene, *Apis mellifera*, der sich als invasive Art rasch ausbreitet. Die Käfer

paaren und vermehren sich innerhalb des Bienenstocks und können somit über verschiedene Kontaminierungswege mit Viren der Honigbiene infiziert werden. Wir präsentieren hier, mit Hilfe von RT-PCR Methoden, den ersten Nachweis von Honigbienenviren im Kleinen Beutenkäfer und in Bienenwachs. Der Kleine Beutenkäfer wurde in Käfigexperimenten mit dem Flügeldeformations-Virus (DWV) infiziert über: (1) Fütterung mit toten Arbeiterinnen mit klinischen Symptomen (deformierte Flügel), (2) Fütterung mit DWV positiver Bienenbrut, (3) Assoziation mit DWV kontaminiertem Wachs und (4) Trophallaxis mit infizierten Arbeiterinnen (Abb. 1). Wir fanden jedoch keinen Hinweis für eine orale Infektion über DWV kontaminierten Pollen (Abb. 1). In 40 % der DWV positiven Käferproben konnte der minus RNA Strang des DWV nachgewiesen werden (Abb. 2), was ein Hinweis dafür ist, dass sich das +RNA Virus im Käfer vermehren kann. Unsere Ergebnisse zeigen zum ersten Mal, dass eine orale Infektion mit Honigbienenviren über Wachs möglich ist. Darüber hinaus ist der Kleine Beutenkäfer ein potentieller biologischer Vektor von Honigbienenviren, was für deren Verbreitung innerhalb und zwischen Völkern relevant sein kann.

***Apis mellifera* / *Aethina tumida* / biologischer Vektor / Deformed Wing Virus / Honigbiene / Kleiner Beutenkäfer**

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