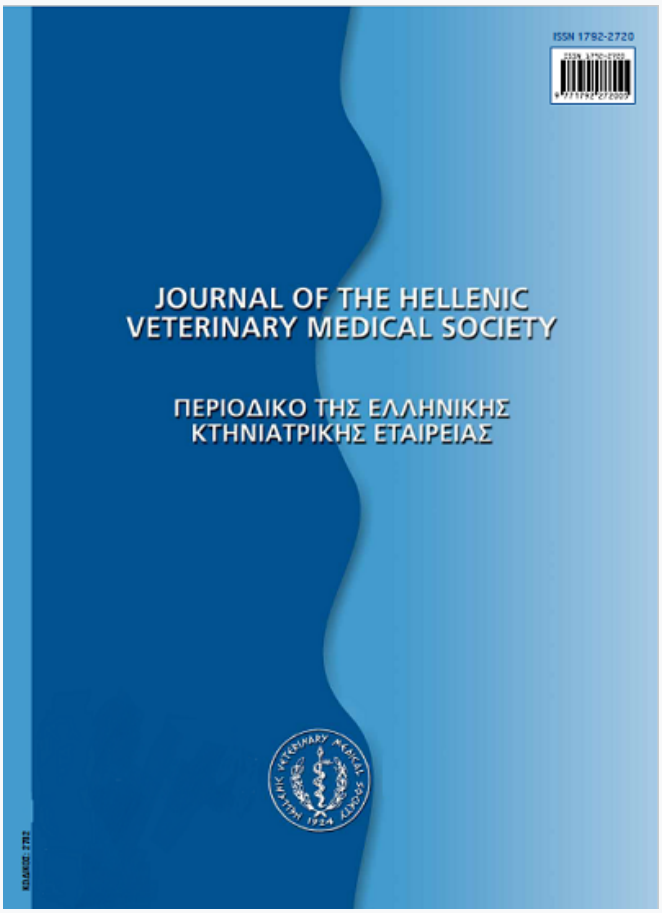
Journal of the Hellenic Veterinary Medical Society

Vol 68, No 4 (2017)



Characterization of a novel recombination event in the Deformed wing bee virus polymerase gene

G GORAS, IGA RUETHER, CH TANANAKI, S GOUNARI, V LIOLIOS, D KANELIS, N ARGENA, M RODOPOULOU, EM KARAZAFIRIS, A THRASYVOULOU

doi: 10.12681/jhvms.16071

To cite this article:

GORAS, G., RUETHER, I., TANANAKI, C., GOUNARI, S., LIOLIOS, V., KANELIS, D., ARGENA, N., RODOPOULOU, M., KARAZAFIRIS, E., & THRASYVOULOU, A. (2018). Characterization of a novel recombination event in the Deformed wing bee virus polymerase gene. *Journal of the Hellenic Veterinary Medical Society*, *68*(4), 661-668. https://doi.org/10.12681/jhvms.16071

J HELLENIC VET MED SOC 2017, 68(4): 661-668
ΠΕΚΕ 2017, 68(4): 661-668

Research article
Ερευνητικό άρθρο

Characterization of a novel recombination event in the Deformed wing bee virus polymerase gene

Goras G.[1], Ruether I.G.A.[1], Tananaki Ch.[1], Gounari S.[2], Liolios V.[1], Kanelis D.1, Argena N.[1], Rodopoulou M.[1], Karazafiris Em.[1] and Thrasyvoulou A.[1]

[1]Laboratory of Apiculture-Sericulture, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Aristotle University, Greece

[2]Institute of Mediterranean Forest Ecosystems and Forest Products Technology, NAGREF, 11528 Athens, Greece

Περιγραφή ανασυνδυασμού στο γονίδιο της πολυμεράσης στον ιό των παραμορφωμένων φτερών μελισσών (DWV)

Γκόρας Γ.[1], Ρούτερ Ι.Γ.Α.[1], Τανανάκη Χρ.[1], Γούναρη Σ.[2], Λιόλιος Β.[1], Κανέλης Δ.[1], Αργενά Ν.[1], Ροδοπούλου Μ.[1], Καραζαφείρης Εμ.[1] και Θρασυβούλου Α.[1]

[1]Εργαστήριο Μελισσοκομίας – Σηροτροφίας, Σχολή Γεωπονίας, Δασολογίας και Φυσικού Περιβάλλοντος, Τμήμα Γεωπονίας, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης

[2]Ινστιτούτο Δασικών Μεσογειακών Οικοσυστημάτω, ΕΛΓΟ «ΔΗΜΗΤΡΑ», Αθήνα

ABSTRACT. Honeybee populations are known to be infected by numerous viruses. Reverse transcription-PCR (RT-PCR) of regions of the RNA-dependent RNA polymerase is often used to diagnose the presence in apiaries and also to classify the type of virus detected.

In this report, through analysis of the RdRp gene, we describe a novel recombination event in the DWV genome. Similarity plot analysis amplified from hundred positive individuals identified a previously undescribed recombination point in the 5' region of the polymerase gene.

Corresponding Author:
Ruether I.G.A.
Laboratory of Apiculture-Sericulture, School of Agriculture, Aristotle's University Farm, 57001 Thermi, Thessaloniki, Greece.
E-mail: irouter@yahoo.gr

Date of initial submission: 9-12-2016
Date of revised submission: 11-1-2017
Date of acceptance: 15-1-2017

To our knowledge this is the first description of recombination in the DWV polymerase gene and highlights the continuous genetic evolution of these viruses.

ΠΕΡΙΛΗΨΗ. Είναι γνωστό ότι οι πληθυσμοί των μελισσών προσβάλονται από πλήθος ιών. Η μέθοδος της RT-PCR **σε περιοχές της** RNA-εξαρτώμενης RNA **πολυμεράσης, χρησιμοποιείται συχνά για τον εντοπισμό των ιών σε μέλισσες αλλά και προκειμένου να ταξινομηθούν αυτοί φυλογενετικά.**

Στην παρούσα έρευνα, μέσω της ανάλυσης του γονιδίου RdRp, περιγράφεται ένας νέος ανασυνδυασμός **στο γένωμα του ιού των παραμορφωμένων φτερών. Μέσω ανάλυσης 100 θετικών δειγμάτων μελισσών, εντοπίστηκε για πρώτη φορά ένας ανασυδυασμός**, **στο 5' άκρο του γονιδίου της πολυμεράσης.**

Αυτή είναι η πρώτη περιγραφή ενός τέτοιου ανασυνδυασμού στο γονίδιο της πολυμεράσης του ιού DW, γεγονός που καταδεικνύει τη συνεχή εξέλιξη των ιών **αυτών**.

Keywords: Deformed wing virus / RdRp / honeybees / Varroa / recombination

Λέξεις κλειδιά: Ιός των παραμορφωμένων φτερών / RdRp / μέλισσες / Βαρρόα / ανασυνδυασμός

INTRODUCTION

Honeybee populations are infected by numerous viruses (Anderson and Trueman, 2000; Chen and Siede, 2007). Single-stranded RNA viruses, infectious to the European honeybee, *Apis mellifera L.* are known to exist at low levels in colonies, with no apparent signs of infection. Among them, Deformed wing virus (DWV) is probably the most widespread of the so-far-described approximately 18 viruses infecting honeybees, transmitted to larvae by the ectoparasitic mite *Varroa destructor* (Ball, 1983; Allen and Ball, 1991; Bailey and Ball, 1991; Lanzi et al., 2006; Berenyi et al., 2007). DW virus appears in coexistence with the ectoparasitic mite *Varroa destructor* (Ball, 1983; Hung et al., 1996; Ball, 1997; Benjeddou et al., 2001;), which is a highly effective vector of DWV transmission among bees, and the virus is able to replicate in mite (Bowen-Walker et al., 1999; Shen et al., 2005; Tentchev et al., 2006). Bee larvae infected during the white-eyed stage of development usually survive the infection initially but latter suffer from deformed wings.

The original host of the mite, the Asian honey bee *Apis cerana Fabr*., does not suffer to appreciable degree by the infestations (Rath and Drescher, 1990). Also it is well documented that Africanized honey bees (*Apis mellifera adansonii* imported into Brazil) also survive and coexist with V. destructor in South America (Rosenkranz, 1999). Similar host – parasite adaptations have also been reported from North Africa (Boecking and Ritter, 1993). The honey bee, *Apis mellifera L*., naturally occurs in Europe, the Middle East, and Africa. However, subspecies of the honey bee have been spread worldwide beyond their natural range, due to economic benefits. More specifically there are several beekeeping units in Greece that imports and merchandise bees (*Apis melifera ligustica*) as pure race or as hybrids (Buckfast bees). Actually Italian bee is the most widely distributed of all races of bees including Greece and many queen producers used it as stock of genetic material. In Europe, after the invasion of Varroa mite it is generally accepted that the mite population must be controlled to avoid viruses' infestation which lead to bee colony collapse.

DWV belongs to the genus *Iflavirus* of the insect picorna-like viruses and serologically is distantly related to Egypt bee virus (Bailey and Woods. 1977; Anderson and Trueman, 2000; Ongus et al., 2004). DWV contains a positive, single-stranded, polyadenylated and monocistronic RNA genome consist of 10,144 nucleotides. The monopartite genome consists of one large, uninterrupted open reading frame encoding the viral polypeptide precursor, which is

post translationally processed by proteases into active proteins.

The N-terminal end of the polypeptide starts with a leader peptide (L protein), followed by the structural proteins VP2, putative VP4, VP1, and VP3. The C-terminal part of the polypeptide contains the non-structural proteins; conserved motifs of the RNA helicase, the putative VPg protein, the C protease and the RNA-dependent RNA polymerase (RdRp) which were predicted in the deduced amino acid sequence (Lanzi et al.,2006).

The RdRp gene is considered as a marker for studies concerning RNA virus classification and evolution (Baker and Schroeder, 2008). The amount of genetic diversity that an RNA virus possesses is a direct result of the virally encoded RNA dependent RNA polymerase and strain recombination (Lohmann et al., 1997; Waters et al., 2007). The domains that have been identified are considered to have important functions with respect to RNA polymerase activity, with studies involving recombinations and amino acid substitutions within particular motifs of these domains having significant impact on the enzymatic activity (Lohmann et al., 1997; Waters et al., 2007; Baker and Schroeder, 2008). It is well estabilised that, in the sequenced regions of the RdRp gene, the DWV genome turned out to be highly conserved, independent of the geographic origins of the honeybee samples: the partial sequences exhibited 98 to 99% nucleotide sequence identity. Substitutions were most frequently observed at the same positions in the various DWV sequences (Berenyi et al., 2007).

To date only two studies were reported, regarding the shortening of abdomens, the discoloring, and the reduction of longevity after infection by DWV (Bailey and Ball, 1991; Yue and Genersch, 2005). To our knowledge this is the first description of recombination in the DWV genome which became dominant, associated with symptoms of infection of the virus independent of wing deformation, and is the only known evidence of infection by the virus to professional beekeepers.

MATERIALS AND METHODS

One hundred samples, contain of fifteen individual adult worker bees each, without signs of deformed wings, were collected from honey bee colonies located in two different areas in northern Greece (fifty different bee colonies each). Bees were immediately dropped into freezer (-20^{o} C), in order to be killed, and RNA was extracted almost immediately.

All selected colonies' samples had never shown symptoms of deformed wings but were chemical treated by beekeepers for previously severe infestation of *Varroa destructor* mites (Tananaki et al., 2014). The bee colonies were examined after the treatment for their level of infestation (Tananaki et al., 2014) and while results suggested that hostparasite co-adaptation ensured survival of both the host and the parasite (Fries et al., 2006; Fries and Bommarco, 2007; Locke et al., 2012), the bee colonies showed characteristic growth retardation and finally collapsed.

Total RNA was extracted from individual samples (homogenized 15 bee-workers each) using the NucleoSpin® RNA II Kit according to the manufacturer's instructions (Macherey-Nagel, Germany).

Five μl of RNA were subjected to cDNA synthesis by Reverse Transcriptase M-MLV (200units/μl, Invitrogen, UK), according to the manufacturer's instructions, using random primers (dN9), (Takara Biomedical group, Shiga, Japan), (50nmol/μl). Three μl of each cDNA were used in subsequent PCR assays.

PCR was carried out using the published primer pairs DWV_FWD (5'-TTTGCAAGATGCTGTATGTGG-3'), DWV_REV (5' GTCGTGCAGCTCGATAGGAT-3') (Nielsen et al., 2008) and DWV8934f (5'-CCTATCGAGCTGCACGACTT-3'), DWV9599r (5'-CCGAGACCTTGTCCAGGTTA-3') (Berenyi et al., 2007). Initially we used the specific published primer pair DWV_FWD-DWV_REV which enabled us to trace this recombination. Then, another published primer pair DWV8934f - DWV9599r was used to cover a more suitable length in RdRp gene, for phylogeny purposes, (total nucleotide length 1021bp) (Kroneman et al., 2011; Ruether et al., 2012; Ruether et al., 2013; Ruether et al., 2014).

Three μl of the reverse transcription product were used for subsequent PCR assays in a total volume of 50μl containing 10x PCR buffer, 2 mM MgCl2, 10 mM dNTPs, 0.5μl of Paq DNA polymerase (Stratagene), (5 U/μl) and 50pmol of each primer pair.

After an initial denaturation step at 95°C for 2min, 40 amplification cycles (denaturation at 95°C for 30s, annealing temperature 54°C for 20s and extension at 72°C for 30s) were performed followed by a final extension step at 72°C for 5 min. In some cases Auto Nested PCR with the same set of primers and under the same conditions for 25 cycles of amplification was applied to increase the sensitivity of the assay (Ruether et al., 2013; Ruether et al., 2014;).

Two independent PCR assays were carried out and amplicons from the two distinct PCR assays were subjected to sequencing. PCR amplicons were purified using the QIAquick® Gel Extraction Kit (Qiagen, Germany) and sequenced bidirectionally at VBC-Biotech (Vienna, Austria). Sequence identity was determined through BLAST (http:www.ncbi.nlm.gov/blast).

RESULTS AND DISCUSSION

All hundred sequenced samples were positive for DWV and shared 99-100% nucleotide similarity thus, for handling ease in further phylogenetic analyses, we randomly selected ten sequences, equally originated from the two different areas. The sequences concerning this report were submitted to GenBank under the accession numbers: DWV_G:3419:KP165134, DWV_G:B1:KP165135, DWV_G:A4:KP165136, DWV_G:B4:KP165137, DWV_G:B7:KP165138, DWV_G:A3:KP165139, DWV_G:KATH1:KP165140, DWV_G:FAG8:KP165141, DWV_G:FAG2:KP165142 and DWV_G:FAG3:KP165143.

Multiple alignments of DWV sequences were generated by ClustalW (available at http://www.ebi.ac.uk/Tools/msa/clustalw2) and the phylogenetic tree was constructed by the neighbor-joining method with 1000 bootstrap replicates in the multiple alignment program MEGA 4.1. All sequences exhibited aberrant clustering on the branch of the tree and it was suspected that those DWV strains had undergone a recombinational event.

The relationship of recombinant strains characterized in the present study, with other strains assessed using sequences available from GenBank.

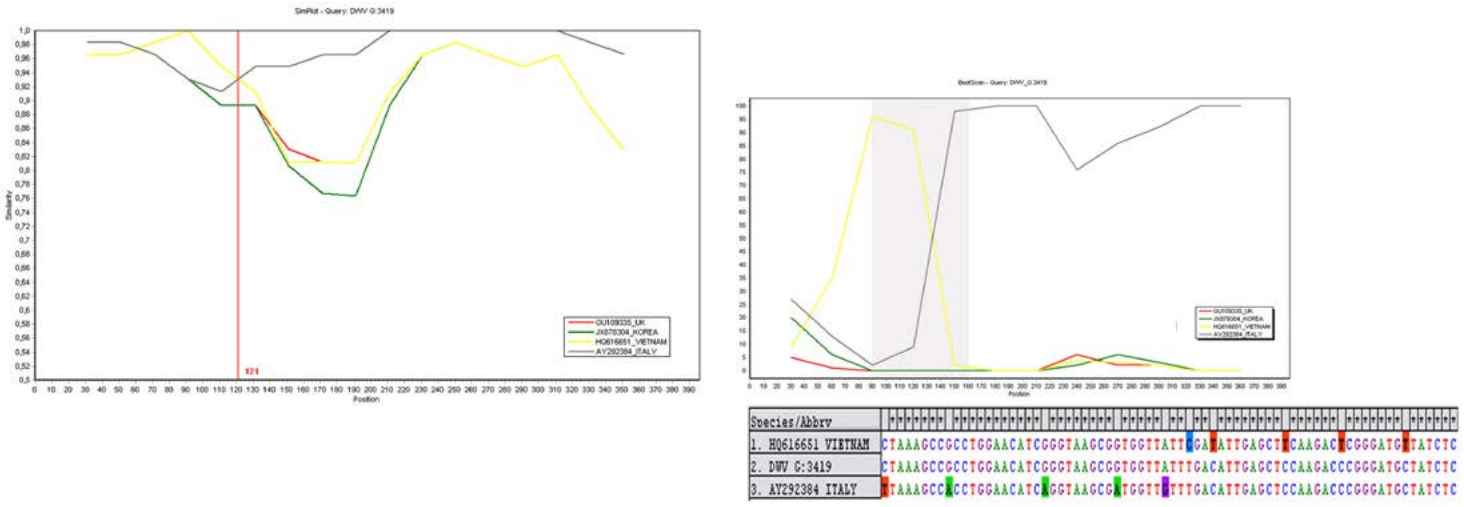


Figure 1. Left: SimPlot analysis for DWV_G:3419 sequence in the 5' region of the polymerase gene (8566-8960nt). Vertical red line indicates the crossover point which was mapped to nucleotide position 8687.

The window size was 100 bp with a step size of 50 bp. The vertical axis indicates the nucleotide identities between the query sequence (DWV_G:3419) and the strains (listed on the window on the right of the figure), expressed as percentages. The horizontal axis indicates the nucleotide positions of the analyzed genome region.

Right: Bootscan analysis of the genomic region involved in the recombination event at nucleotide position 8687 of DWV_G:3419 strain in the 5' region of the polymerase gene (8566-8960nt).

The shaded area represents the point of recombination and underneath, are presented the three DNA fragments aligned together at the point of recombination.

To identify the putative parent-like strains and potential recombination sites, phylogenetic profile analysis was performed using SimPlot program (Ruether et al., 2012; Ruether et al., 2013; Ruether et al., 2014).

The plots of the nucleotide sequences are depicted in (Fig. 1-left). Since the strains shared 99-100% nucleotide similarity, we used one of them, strain DWV_G:3419 for the analysis. When a similarity plot for the representative strain (DWV_G:3419) was generated, with strains available from GenBank, a recombination breakpoint, which was mapped to nucleotide position 8687 (with respect to the Deformed wing virus isolate PA, accession no AY292384) of the sequence alignment was visible. These findings were further confirmed by boot scanning of the same genome sequences, demonstrating higher levels of phylogenetic relatedness between the DWV_G:3419 genome sequence and the HQ616651_VIETNAM and AY292384_ITALY genome sequence on the upstream and downstream side of the recombination site, respectively (Fig.1-right).

Finally, phylogenetic analyses were conducted using two neighbor-joining phylogenetic trees (1000

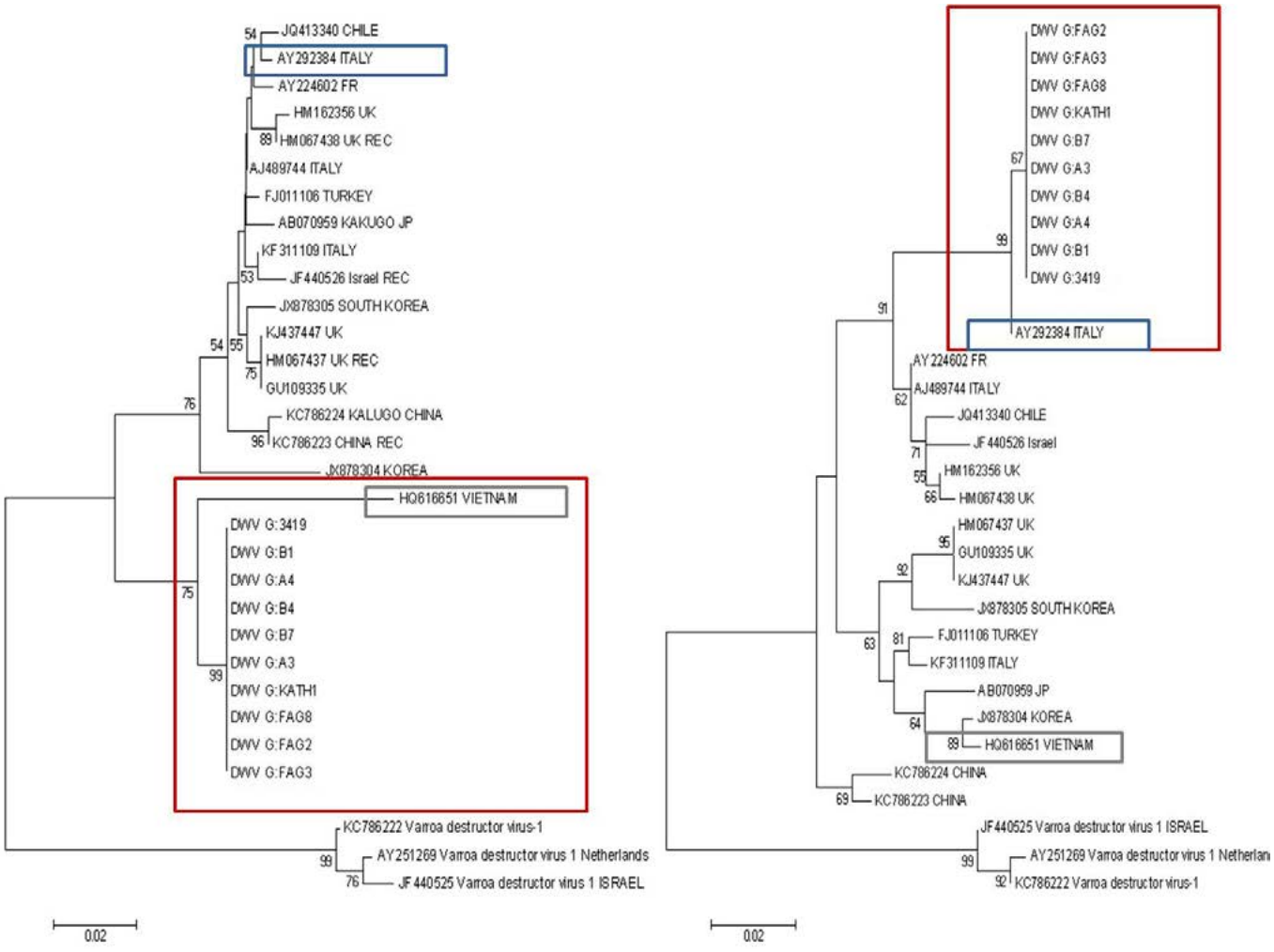


Figure 2. Neighbour-joining phylogenetic trees based on sequences before and after the identified recombination site. Left: the examined sequences (8566-8687nt) are most similar to the strain HQ616651_VIETNAM and clusters on a separate branch. Right: the examined sequences (8687-9587nt) clusters with the AY292384_ITALY strain on a separate branch with bootstrap value 100. Bootstrap values of less than 50% are not shown.The shaded area represents the point of recombination and underneath, are presented the three DNA fragments aligned together at the point of recombination.

bootstrap replicates in the multiple alignment program MEGA 4.1.), based on sequences found either side of the identified recombination site, were compiled to confirm recombinant strains detection (fig.2). Moreover, we used partially sequenced DWV strains that were genetically close according to BLAST clustering. Our initial phylogenetic analysis revealed that these strains were indeed recombinant.

DWV genus is highly genetically diverse and this is, in part, maintained by homologous recombination. Studies have reported single-stranded RNA viruses that hypothetically evolve via epochal evolution; these include influenza virus (Koelle et al., 2006; Van Nimwegen, 2006), NoVs (Siebenga et al., 2007; Lindesmith et al., 2008) and others. In general, epochal evolution is a process whereby periods of stasis defined as no differences in phenotype (epochs) are observed, followed by rapid bursts of evolution (innovations) (Donaldson et al., 2008). These observations raise questions about the biological properties of the recombinants and the mechanisms of their dissemination. Two plausible explanations may be given: a) similar to other RNA viruses they may undergo an epochal evolution driven by the host immunity, where herd immunity could be a selection force in the evolution of these viruses, in which new antigenic variants emerge and become predominant because of the lack of herd immunity (Donaldson et al., 2008), b) the continual appearance of new variants and recombinant is a result of colonial expansion from quasi-species by fitness for more efficient viral replication, virulence, and broader host range. Thought direct evidence for these two plausible explanations is still lacking.

Interestingly, in this study, all recombinant strains isolated from asymptomatic individuals to known symptom of deformed wings, showed an unexplained behavior - growth retardation- which could be considered a new symptom of infection. Theoretically, herd immunity would have developed within this bee population, and so to avoid extinction, the virus had to either evolve to evade herd immunity or evolve to infect new bee populations.

Moore *et al*. (Simmonds, 2006) demonstrated that the evolution of DWV-related viruses included recombination of three genome 'modules' and had identified two novel recombinants. This report is the first description of recombination in the DWV polymerase gene and to our knowledge to date no intergenogroup recombinant strain has been identified. Recombination between genomes occurs frequently and is another important feature of the RNA virus evolution. Recombination may influence virulence as well as being an essential mechanism for maintenance of the virus in the population. Spread and occurrence of recombinant strains in symptomatic individuals validate their infectivity in hosts and shows that recombination does not prevent virulence (Koonin and Dolja, 1993; Donaldson et al., 2008; Moore et al., 2011). Given the fact that these viruses belong to the family picorna-like viruses in which belong many other viruses that have an adverse effect on humans (enteroviruses, hepatitis A viruses, etc.) these results highlight the continuous genetic evolution of these viruses. Comparing the evolutionary profiles of these different viruses, will likely provide significant insights into how the DW viruses became so successful at evading the immune system by either escape or by penetrating previously naive populations or both (Koonin and Dolja, 1993; Simmonds, 2006).

CONCLUSIONS

To conclude, these findings can also contribute to form the basis for improved understanding of the role of DWV and recombinants, thus in the pathogenesis of deformed wing diseases of honey bees.

As recombination allows the virus to increase its genetic fitness and to evolve, to spread in the population and probably to escape the host immune response, our findings suggest that the capacity for genetic changes displayed by the DWVs will continue to generate new recombination types.

REFERENCES

Allen M and Ball BV (1996) The incidence and world distribution of honeybee viruses. Bee World 77:141-162.

Anderson DL and Trueman JWH (2000) *Varroa jacobsoni* (Acari:-Varroidae) is more than one species. Exp Appl Acarol 24:165-189.

Bailey L and Ball BV (1991) Honey bee pathology, 2nd edition. Academic Press London, United Kingdom 10-30,97-104.

Bailey L and Woods RD (1977) Two more small RNA viruses from honey bees and further observations on sacbrood and acute bee paralysis viruses. J Gen Virol 37:175-182.

Baker AC and Schroeder D (2008) The use of RNA-dependent RNA polymerase for the taxonomic assignment of Picorna-like viruses (order Picornavirales) infecting *Apis mellifera* L. populations. Virol J 22:5-10.

Ball BV (1997) Varroa and viruses. In: P. Munn and R. Jones (ed.),-Varroa! Fight the mite. International Bee Research Association, Cardiff,Wales, United Kingdom. pp 11–15.

Ball BV (1983) The association of *Varroa jacobsoni* with virus diseases of honey bees. *In Varroa jacobsoni* Oud affecting honey bees: present status and needs Edited by: Cavallovo R. *Rotterdam*: Commission of the European Communities 21-23.

Benjeddou M, Leat N, Allsopp M, Davison S (2001) Detection of acute bee paralysis virus and black queen cell virus from honeybees by reverse transcriptase PCR. Appl. Environ. Microbiol 67:2384–2387.

Berényi O, Bakonyi T, Derakhshifar I, Köglberger H, Topolska G, Ritter W, Pechhacker H, Nowotny N (2007) Phylogenetic analysis of deformed wing virus genotypes from diverse geographic origins indicates recent global distribution of the virus. Appl Environ Microbiol 73(11):3605 - 11.

Boecking O, Ritter W (1993) Grooming and removal behavior of *Apis mellifera intermissa* in Tynesia against *Varroa jacobsoni*, J Apic. Res. 32:127-134.

Bowen-Walker PL, Martin SJ, Gunn A (1999) The transmission of deformed wing virus between honeybees (*Apis mellifera* L.) by the ectoparasitic mite *Varroa jacobsoni* Oud. J Invertebr Pathol 73:101–106.

Chen Y and Siede R (2007) Honeybee viruses. Adv Virus Res 70:33-80.

Donaldson F, Lindesmith LC, Lobue AD, Baric RS (2008) Norovirus pathogenesis: mechanisms of persistence and immune evasion in human populations. Immunol Rev 225:190-211.

Fries I, Bommarco R (2007) Possible host–parasite adaptations in honey bees infested by *Varroa destructor* mites. Apidologie 38(6):525–533.

Fries I, Imdorf A, Rosenkranz P (2006) Survival of mite infested (*Varroa destructor*) honey bee (*Apis mellifera*) colonies in a Nordic climate. Apidologie 37:564–570.

Hung ACF, Shimanuki H, Knox DA (1996) The role of viruses in bee parasitic mite syndrome. Am Bee J 136:731–732.

Koelle K, Cobey S, Grenfell B, Pascual M (2006) Epochal evolution shapes the phylodynamics of interpandemic influenza A (H3N2) in humans. Science 314:1898–1903.

Koonin EV and Dolja VV (1993) Evolution and taxonomy of positive strand RNA viruses: Implications of comparative analysis of amino acid sequences. Crit Rev Biochem Mol Biol 28:375-430.

Kroneman A, Vennema H, Deforche K, v d Avoort H, Peñaranda S, Oberste MS, Vinje J, Koopmans M (2011) An automated genotyping tool for enteroviruses and noroviruses. J Clin Virol 51(2):121-125.

Lanzi G, de Miranda JR, Boniotti MB, Cameron CE, Lavazza A, Capucci L, Camazine SM, Rossi C (2006) Molecular and biological characterization of deformed wing virus of honeybees (*Apis mellifera* L.). J Virol 80:4998-5009.

Lindesmith LC, Donaldson EF, Lobue AD, Cannon JL, Zheng DP. Vinje J, Baric RC (2008) Mechanisms of GII.4 norovirus persistence in human populations. PLoS Med 5(2): e31.

Locke B, Conte YL, Crauser D, Fries I (2012) Host adaptations reduce the reproductive success of *Varroa destructor* in two distinct European honey bee populations. Ecol Evol 2(6):1144-50.

Lohmann V, Korner F, Herian U, Bartenschlager R (1997) Biochemical properties of Hepatitis C virus NS5B RNA-dependent RNA-polymerase and identification of amino acid sequence motifs essential for enzymatic activity. J Virol 71:8416-8428.

Moore J, Jironkin A, Chandler D, Burroughs N, Evans DJ, Ryabov EV (2011) Recombinants between Deformed wing virus and Varroa destructor virus-1 may prevail in *Varroa destructor*-infested honeybee colonies. J Gen Virol 92(Pt 1):156-61.

Nielsen SL, Nicolaisen M, Kryger P (2008) Incidence of acute bee paralysis virus, black queen cell virus, chronic bee paralysis virus, deformed wing virus, Kashmir bee virus and sacbrood virus in honey bees (*Apis mellifera*) in Denmark. Apidologie 39:310-314.

Ongus JR, Peters D, Bonmatin JM, Bengsch E, Vlak JM, van Oers MM (2004) Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite *Varroa destructor*. J Gen Virol 85:3747-3755.

Rath W and Drescher W (1990) Response of *Apis cerana* Fabr. towards brood infested with *Varroa jacobsoni* Oud. And infestation rate of colonies in Thailand, Apidologie 21:311-321.

Rosenkranz P (1999) Honey bee (*Apis mellifera* L.) tolerance to *Varroa jacobsoni* Oud. In South America, Apidologie 30:159-172.

Ruether IGA, Tsakogiannis D, Pliaka V, Kyriakopoulou Z, Krikelis A, Gartzonika C, Leveidiotou-Stefanou S, Markoulatos P (2012) Molecular characterization of a new intergenotype Norovirus GII recombinant. Virus Genes 44(2):237-43.

Ruether IGA, Tsakogiannis D, Kyriakopoulou Z, Dimitriou TG, Papamichail C, Gartzonika C, Leveidiotou-Stefanou S, Markoulatos P (2013) Circulation of intergenotype recombinant noroviruses GII9/GII6 from 2006 το 2011 in central Greece. Virus Genes 48(1):23-31.

Ruether IGA, Dimitriou TG, Tsakogiannis D, Kyriakopoulou Z, Amoutzias GD, Gartzonika C, Levidiotou-Stefanou S, Markoulatos P (2014) Characterization of novel intergenogroup and intergenotype recombinant noroviruses from central Greece. Mol Cell Probes 28(4):204-10.

Shen M, Yang X, Cox-Foster D, Cui L (2005) The role of varroa mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. Virology 342:141–149.

Siebenga JJ, Vennema H, Renckens B, de Bruin E, van der Veer B, Siezen RJ, Koopmans M (2007) Epochal evolution of GGII.4 norovirus capsid proteins from 1995 to 2006. J Virol 81(18):9932-9941.

Simmonds P (2006) Recombination and selection in the evolution of picornaviruses and other Mammalian positive-stranded RNA viruses. J Virol 80:11124-11140.

Tananaki Ch, Goras G, Huggett N, Karazafiris E, Dimou M, Thrasyvoulou A. (2014) Evaluation of the impact of Exomite Pro on Varroa mite (*Varroa destructor*) populations and honeybee (*Apis mellifera*) colonies: efficacy, side effects and residues. Parasitol Res 113(4):1251-9.

Tentchev D, Gauthier L, Bagny L, Fievet J, Dainat B, Cousserans F, Colin M E, Bergoin M (2006) Comparative analysis of deformed wing virus (DWV) RNA in *Apis mellifera* L. and *Varroa destructor*. Apidologie 37:41–50.

Van Nimwegen E (2006) Epidemiology. Influenza escapes immunity along neutral networks. Science 314:1884-1886.

Waters A, Coughlan S, Hall WW (2007) Characterisation of a novel recombination event in the norovirus polymerase gene. Virology 363(1):11-4.

Yue C and Genersch E (2005) RT-PCR analysis of Deformed wing virus in honeybees (*Apis mellifera*) and mites (*Varroa destructor*). J Gen Virol 86:3419-3424.