A DISSERTATION

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Identification and Characterization of *Diadegma fenestrale* Ichnovirus (DfIV) and Plasticity of Its Genome Expression Patterns in Parasitized Hosts

감자뿔나방살이자루맵시벌 이크노바이러스의 동정 및 특성구명과
피기생기주내 바이러스 유전체 발현의 가소성

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ABSTRACT

Parasitoids are found in several insect orders. Among them, Hymenopteran parasitoids are most common paticually Ichneumonoidea. Ichneumonoidea is one of the largest superfamily in Hymenoptera and has four families containing over 60,077 species. The rich species abundance may be achieved with accompanying symbiotic parasitic factors including symbiotic virus, polydnavirus (PDV). PDV belonging to Polydnaviridae and is classified into two groups based on their parasitoid host, Bracovirus (BV); Braconidae and Ichnovirus (IV); Ichneumonidae. This study reports a novel PDV from an endoparasitoid wasp, *Diadegma fenestrale* (Hymenoptera: Ichneumonidae: Campopleginae). The viral particles were detected in female reproductive organ and showed the typical IV morphology of double membrane structure and segmented genome. This virus was named as *D. fenestrale* ichnovirus (DfIV). A total of 65 discrete genome segments were separated from the viral DNA extract, and the entire DfIV genome (247,191 bp) was subsequently sequenced and annotated. Among the 65 segments, 62 segments showed a high similarity to *Hyposoter fugitivus* ichnovirus (HfIV) as determined by BLAST analysis. The average GC contents of DfIV genome was 43.3%. A total of 99 open reading frames (ORFs) were predicted as follows:

40 ORFs of repeat element protein (*rep*), 12 ORFs of cysteine motif protein (*cys motif*), 8 ORFs of viral ankyrin (*vankyrin*), 6 ORFs of viral innexin (*vinnexin*), 2 ORFs of polar residue-rich, 1 ORF of N gene and 30 ORFs of other unassigned genes. The potato tuber moth (PTM, *Phthorimaea operculella*, Lepidoptera: Gelechiidae) and the diamondback moth (DBM, *Plutella xylostella*, Lepidoptera: Plutellidae) were parasitized by *D. fenestrale*. Nevertheless, based on the oviposition and survival rate, it appeared that *D. fenestrale* prefers PTM to DBM as hosts. Moreover, DfIV genes were more widely expressed in PTM than DBM after parasitized by *D. fenestrale*, particularly within a day after parasitized. These initial responses were very important to determine the success or fail of parasitism. In addition, a large number of DfIV genes were expressed only in PTM and these genes exhibited differential expression patterns in two lepidopteran hosts. This finding suggests that the DfIV genome expression plasticity depends on the lepidopteran host species and post parasitization time lapse, perhaps contributing to the enhancement of the parasitoid survival rate. Such host-specific DfIV gene expression may play a crucial role in shaping the symbiotic and coevolutionary relationship between the PDV and the parasitoid. These newly identified DfIV genes could be apply for various research fieds.

Key words: *Diadegma fenestrale, Diadegma fenestrale* ichnovirus (DfIV), polydnavirus, genome, *Phthorimaea operculella, Plutella xylostella,* deep sequencing, gene expression,

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Footnote

Abbreviation used: DfIV, *Diadegma fenestrale* ichnovirus; PDV, polydnavirus; IV, ichnovirus; BV, bracovirus; PTM, potato tuber moth; DBM, diamondback moth; *rep*, repeat element protein gene; *cys-motif*, cysteine motif protein gene; *cys-rich*, cysteine rich protein gene; *vankyrin*, viral ankyrin; *vinnexin*, viral innexin; NGS, next generation sequencing; RNA-seq, whole transcriptome shotgun sequencing; qrt-PCR, quantitative real time polymerase chain reaction; BLAST, basic local alignment search tool; DELTA BLAST, domain enhanced lookup time accelerated BLAST; ORF, open reading frame; CDD, conserved domain database; EtBr, ethidium bromide; TEM, transmission electron microscopy; RPKM, reads per kilo base per million; GO, gene ontology; HARC, Highland Agriculture Research Center; JH, juvenile hormone; JHE, juvenile hormone esterase; AsIV, *Apophua simplicipes* ichnovirus; CcIV, *Campoletis chlorideae* ichnovirus; CsIV, *Campoletis sonorensis* ichnovirus; DsIV, *Diadegma semiclausum* ichnovirus; GfIV, *Glypta fumiferanae* ichnovirus; HdIV, *Hyposoter didymator* ichnovirus; HfIV, *Hyposoter fugitivus* ichnovirus; TrIV, *Tranosema rostrale* ichnovirus; CcBV, *Cotesia congregata* bracovirus; CgBV, *Cotesia glomerata* bracovirus, CmBV, *Cotesia melanoscela* bracovirus; CpBV, *Cotesia plutellae* bracovirus; CvBV, *Cotesia vestalis* bracovirus; MdBV, *Microplitis demolitor* bracovirus

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Introduction

The endoparasitoid wasp *Diadegma fenestrale* (Hymenoptera: Ichneumonidae: Campopleginae) was first reported in *Diadegma* genus in Korea (Choi et al., 2013). The genus represents a large group of parasitoid wasps with 201 species known to occur worldwide, which have a single host or a wide range of hosts (Yu and Horstmann, 1997). These wasps inject their eggs into a host, where they hatch and subsequently feed on the host. For successful parasitism, wasps cause changes in their hosts' conditions in support of the developing parasitoid larvae. For this purpose, female wasps introduce, the polydnavirus (PDV, *Polydnaviridae*, double stranded DNA virus), a symbiotic virus (Etebari et al., 2013; Etebari et al., 2011; Huang et al., 2009b). PDVs are genetic symbionts of some endoparasitoid wasps, which exhibit koinobiotic life histories (Pennacchio and Strand, 2006). Parasitization by these wasps usually induces significant immunosuppression and altered development of their hosts and PDVs have been maintained as important contributors to these parasitic effects on host immunity and development (Dupuy et al., 2006). More than 30,000 species of parasitoid wasp are thought to carry their own PDVs, although only about 50 species have been described systematically (Dupuy et al., 2006; Lapointe et al., 2007). PDVs are divided into ichnovirus (IV) and bracovirus (BV) depending on host insect family and viral morphology (Federici and Bigot, 2003). BVs typically enclose one or more barrel-shaped nucleocapsids per virion surrounded by a single envelope, whereas IVs typically contain one lenticular nucleocapsid per virion surrounded by two membranes (Webb, 1998.). BVs are distributed across six subfamilies of Braconidae (i.e., Cardiochilinae, Cheloninae, Khoikhoiinae, Mendesellinae, Microgastrinae and Miracinae). Most of IVs are found in wasps of the subfamily Campopleginae, whereas some IV found in the subfamily Banchinae was reported (Djoumad et al., 2013b). However, the information and functional research on IV genomes are much less than those on BVs.

As described above, *D. fenestrale* is known to parasitize more than two lepidopteran hosts, including potato tuber moth (PTM, *Phthorimaea operculella*, Lepidoptera: Gelechiidae) and the

diamondback moth (DBM, *Plutella xylostella*, Lepidoptera: Plutellidae) (Kim et al., 2012). Nevertheless, the emergency rate of *D. fenestrale* from field-collected PTM larvae was more than two-fold higher than that of DBM (Kim et al., 2012). Therefore, this finding led to ask following questions: why does *D. fenestrale* prefer PTM to DBM and why is the parasitism success rate higher in PTM? The molecular mechanisms for successful parasitism or host preference of parasitoids have not been well elucidated. The symbiotic virus, PDV was reported as one of the factors for successful parasitism (Bae and Kim, 2004; Espagne et al., 2005) and host range determination (Cui et al., 2000). In an attempt too understand the molecular basis of the host preference or parasitism success rate of *D. fenestrale*, I primarily focused on the characterization of PDV.

In this study, I completely analyzed the *D. fenestrale* Ichnovirus (DfIV) genome by NGS with capillary sequencing and then annotated putative viral genes. To investigate the differences in parasitism rate of *D. fenestrale* between two lepidopteran hosts, the deep sequencing-based transcriptional profilings of DfIV and its hosts over the time course of parasitization, were carried out for parasitized or non-parasitized larval samples of PTM and DBM. This study would contribute to the understanding of host-specific gene expression patterns of PDV.

Literature Review

1. Parasitoid and polydnavirus

Parasitoids are found in several insect orders (Diptera, Coleoptera, Lepidoptera, Trichoptera, Neuroptera, Strepsiptera, and Hymenoptera)(Pennacchio and Strand, 2006). Especially, Hymenopteran parasitoids are most common because recent estimates indicate that 10% to 20% of all insects may be parasitoid wasps (Godfray, 1994; Pennacchio and Strand, 2006; Whitfield, 2003). Ichneumonoidea is one of the largest superfamily in Hymenoptera and has four families (Ichneumonidae, Braconidae, Eoichneumonidae and Praeichneumonidae) containing over 60,077 species (Taxapad 2012, Ichneumonoidea 2011, www.taxapad.com) (Davis et al., 2010; Kopylov, 2012; Pennacchio and Strand, 2006). The rich species abundance may be achieved by accompanying symbiotic parasitic factors, including PDVs (Dupuy et al., 2006; Pennacchio and Strand, 2006; Turnbull and Webb, 2002). PDV, belonging to Polydnaviridae, is classified into two groups based on their parasitoid host: BV, Braconidae vs. IV, Ichneumonidae (Fig. 1) (Bezier et al., 2009b; Webb, 1998.). PDV-carrying wasp lineages are also ancient, with the fossil record demonstrating their existence over at least 60 million years (Whitfield, 2000). Although the two families are related, their common ancestors do not carry PDVs. Therefore, even though there was no clearly supported evolutionary pathway elucidating the evolutionary origin of PDVs, some paper suggests that the origins of BVs and IVs are distinct and that PDVs are paraphyletic (Turnbull and Webb, 2002). Most BVs have enveloped bacilliform particles and these resemble baculovirus and nudivirus virions (Federici and Bigot, 2003). Characterization of viral RNA polymerase and structural components of BVs particles related most closely to those of nudiviruses (Bezier et al., 2009a). IVs have enveloped spindle-shaped particles that resemble virions of ascoviruses (Federici and Bigot, 2003). Molecular evidence supported that IVs originated from ascoviruses (Bigot et al., 2008; Volkoff et al., 2010). However, their real evolution has been processed with parasitoids.

The genus *Diadegma* (Hymenoptera: Ichneumonidae, Campopleginae) represents a large group of parasitoid wasps with 201 species known to occur worldwide (Yu and Horstmann, 1997). *Diadegma* adult females parasitize larvae of various lepidopteran species. *D. fenestrale* has a wide host range, as Hardy reported that *D. fenestrale* attacked 24 species of lepidopteran and a coleopteran and described it as 'very polyphagous' (Hardy, 1938). At least two families (Gelechiidae and Plutellidae) (Azidah et al., 2000; Choi et al., 2013; Rondon, 2010) were confirmed as their hosts in Korea, such as *Phthorimaea operculella*, *Scrobipalpa salinella* and *P. xylostella* (Kim et al., 2012). *D. fenestrale* was first collected in Jeju, 2009, Korea and reported in 2013. *D. fenestrale* was the first reported *Diadegma* genus in Korea and its Korean name is 감자뿔나방살이자루맵시벌 (Choi et al., 2013). In many cases, *D. fenestrale* was studied with *D. semiclausum*, which is a well known biological control agent against *P. xylostella*. They are morphologically very close and share a common host, *P. xylostella.* Therefore, PCR-based species identification methods of these two *Diadegma* species were developed and molecular phylogeny study was conducted (Wagener et al., 2004; Wagener et al., 2006). *D. fenestrale* was also used as a reference species of *D. semiclausum* in the evolutionary study of *Diadegma* genus because the two species can be interbred (Andrew et al., 2009) but they have some different life style, including different host range (Gols et al., 2008). Because of these characteristics, their basic biology and developmental characteristics have been studied already (Gols et al., 2008; Kim et al., 2012). However, their IVs have not been examined in detail. Only *D. semiclausum* ichnovirus (DsIV) has been reported and some genes are known to contribute to lepidopteran host immune suppression (Etebari et al., 2011; Huang et al., 2008; Huang et al., 2009b).

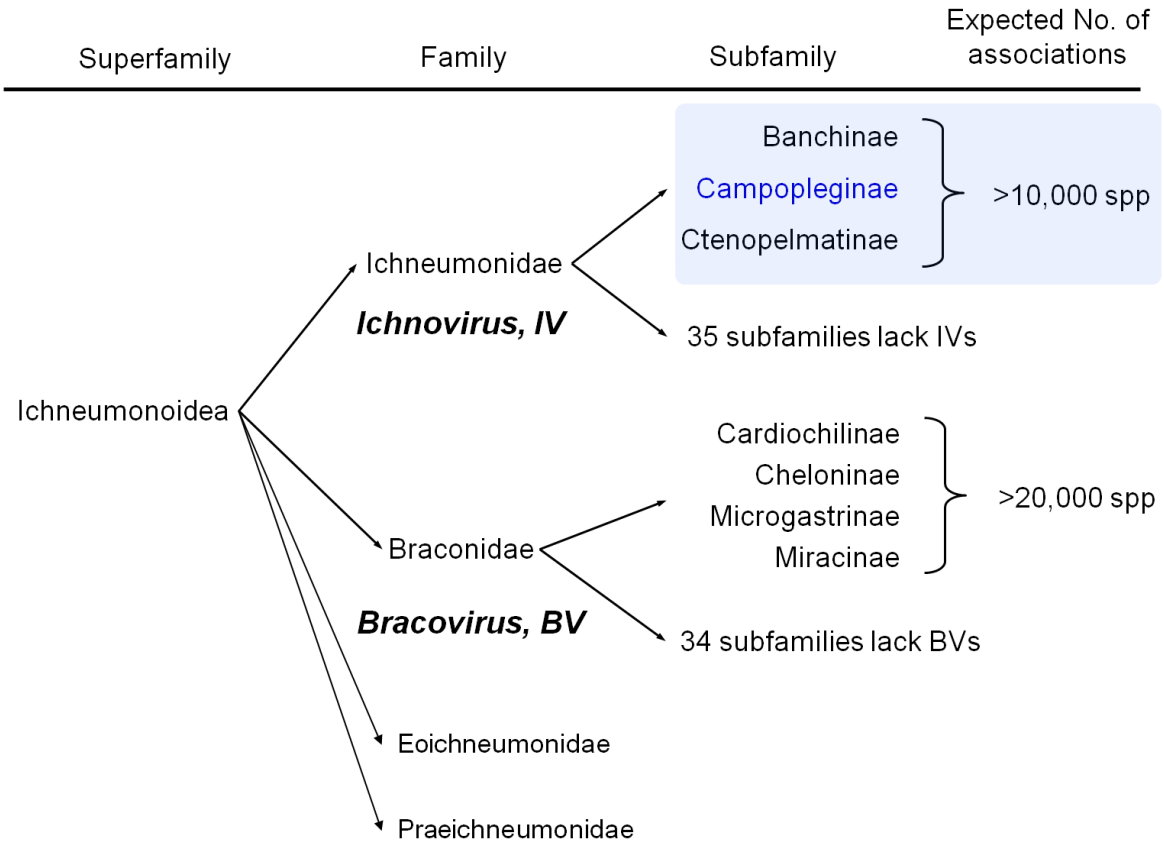


Fig. 1. Phylogenetic relationships showed within the Ichneumonoidea superfamily with PDVs. IV association is limited to three subfamilies of icheneumonid wasps and BV is limited to the microgastroid complex, composed of four subfamilies. This figure was modified from the article "polydnavirus origins and evolution" (Turnbull and Webb, 2002).

2. Polydnavirus

2.1. Lifecycle of polydnavirus

Two PDVs (i.e., BVs and IVs) have differentially evolved with their parasitoid hosts. However, they employ both parasitoids and parasitoids' hosts, such as lepidopteran caterpillar, as their hosts (Kroemer and Webb, 2004). PDV life cycles have been described as having "two arms" (Stoltz, 1993). Virus replication and vertical transmission occur only in the wasp, whereas viral genes disrupting the physiology of the parasitized lepidopteran host function only in the other "arm" of life cycle. Although the two PDV genera have similar life cycles and genomic organization, the viruses are morphologically and genetically distinct, suggesting that the genomic similarities result from selection pressures imposed by their unusual life cycles. PDVs replicate from proviral DNA in specialized cells of the wasp calyx cells and replication is first detected in the late pupal stage with virus released from calyx cells by budding (IV) (Volkoff et al., 1995) or cell lysis (BV) (Stoltz et al., 1976) and accumulated to high concentrations in the oviduct lumen. When the wasp parasitizes its insect hosts, usually lepidopteran larvae, virus is delivered with the wasp egg. The virus enters lepidopteran cells, where a host-specific subset of viral genes is expressed without virus replication (Theilmann and Summers, 1986). Viral gene expression inhibits the host immune responses to the parasitoid egg, thereby enhancing wasp survival (Asgari et al., 1996; Cui et al., 2000). Virus transmission to next generations is asured by stable integration of proviral DNA segments in the wasp genome (Savary et al., 1997; Savary et al., 1999).

2.2. Genome of polydnavirus

The PDV genome consists of multiple circular double stranded DNA segments, ranging in size from 2 to 42 kb (Kroemer and Webb, 2004). The number of genome segments and their size distribution vary among PDVs. The estimated size of characterized PDV genomes ranges from 187 to 567 kb (Dupuy et al., 2006; Espagne et al., 2004; Webb, 1998.; Webb et al., 2006). PDV genes are classified into three groups according to the host specificity of their expression (Webb, 1998.).

Genes expressed exclusively in the wasp or in the lepidopteran host are designated as class I and class II genes, respectively, whereas class III genes are expressed in both hosts. The genomes of the *Apophua simplicipes* ichnovirus (AsIV), *Campoletis sonorensis* ichnovirus (CsIV, type species in genius, IV), *Glypta fumiferanae* ichnovirus (GfIV), *Hyposoter didymator* ichnovirus (HdIV), *Hyposoter fugitivus* ichnovirus (HfIV), *Tranosema rostrale* ichnovirus (TrIV), *Cotesia congregata* bracovirus (CcBV), *Cotesia vestalis* bracovirus (CvBV) and *Microplitis demolitor* bracovirus (MdBV) were recently sequenced (Chen et al., 2011; Choi et al., 2009c; Djoumad et al., 2013b; Lapointe et al., 2007; Rasoolizadeh et al., 2009; Tanaka et al., 2007; Volkoff et al., 2010) and compared with respect to their organization and gene content (Espagne et al., 2004; Stoltz and Xu, 1990; Tanaka et al., 2007; Volkoff et al., 2010; Webb et al., 2006). Partial genome sequences or some gene families have been reported for some PDVs, such as *Diadegma semiclausum* ichnovirus (DsIV) and *Campoletis chlorideae* ichnovirus (CcIV) (Etebari et al., 2011; Tian et al., 2007). Until now, large numbers of PDV genome have not been analyzed as in the case of *Cotesia melanoscela* bracovirus (CmBV, type species in genius, BV).

These IVs and BVs genomes contain a few shared gene families: a preliminary comparison of available sequence data from several PDV species suggested that the gene families identified so far are well conserved within the IV and BV taxa (Webb et al., 2006). However, IVs and BVs are known to have their own evolutionary lineage with their parasitoids (Turnbull and Webb, 2002).

Chapter Ⅰ.

Characterization of *Diadegma fenestrale* Ichnovirus (DfIV)

Abstract

A novel DfIV was discovered from the reproductive organ of *D. fenestrale* female. DfIV was observed in the ovary, particulary in calyx, and conformed to the typical IV morphology of double membrane structure and segmented genome. A total of 65 genome segments were identified and the entire DfIV genome (247,191 bp) was sequenced and annotated. Among the 65 segments, 62 segments showed a high similarity to HfIV as determined by BLAST analysis. The relative abundance of DfIV genome segments varied. The average GC contents of DfIV genome was 43.3%. Based on BLAST analysis, a total of 99 ORFs were predicted as follows: repeat element protein (*rep*; 40), cysteine motif protein (*cys-motif*; 12), viral ankyrin (*vankyrin*; 8), viral innexin (*vinnexin*; 6), polar residue rich (2), N gene (1) and other genes (30). Based on these genes' phylogenetic relationship, DfIV was confirmed as a typical IV. This is the first reported IV from *Diadegma* genus at a genome level.

Key words: *D. fenestrale, D. fenestrale* ichnovirus (DfIV), genome, *P. operculella*, *P. xylostella*

1. Introduction

PDV (Polydnaviridae) is an insect virus symbiotic to some hymenopteran insects (Stoltz and Vinson, 1979). It is divided into two genera, BV and IV, by its different insect host families, viral morphology and gene contents (Webb, 1998.) According to the International Committee on Taxonomy of Viruses (http://www.ictvonline.org/index.asp), 32 species of BVs and 21 species of IVs were recorded. PDVs genomes are double-stranded DNA and segmented, ranging from 187 to 567 kb (Dupuy et al., 2006; Espagne et al., 2004; Webb et al., 2006). These PDVs replicate viral particles in parasitoid ovary particularly, calyx (Stoltz, 1993) and they are accumulated in the oviduct lumen and transferred into hosts along with parasitoid eggs (Norton et al., 1975). The parasitized hosts disrupted their immune system and altered physiological status favorable for parasitoid survival and development (Huang et al., 2009b; Strand and Burke, 2012). This is because PDVs have their functional genes for hosts' physiology manipulation, such as transcription inhibition (Barandoc and Kim, 2009; Shelby et al., 1998). Among these, only a few genes have been identified in their physiological functions. Several research groups have interest in its genomic composition to isolate functional genes and PDV genome itself (Barat-Houari et al., 2006; Choi et al., 2009c; Lapointe et al., 2007; Tanaka et al., 2007).

The genus *Diadegma* represents a large group of parasitoid wasps with 201 species known to occur worldwide including Korea (Choi et al., 2013). Some *Diadegma sp.* studied for biological control as a endoparasite against lepidopteran pests such as *Plutella xylostella* (Xu et al., 2001) and some IVs reported from parasitoid (Etebari et al., 2011; Krell, 1987). *D. fenestrale* is a single species in the genus *Diadegma* which reported in Korea (Choi et al., 2013). *D. fenestrale* has two main hosts, PTM and DBM. As described above, I want to understand this host preference or successful parasitism rate of *D. fenestrale* in the fields, I focused on the PDV. However untill now, any information of PDV from *D. fenestrale* was not reported. Therefore, I try to characterize PDV from *D. fenestrale*.

2. Materials and Methods

2.1. Insects

2.1.1. Parasitoid

D. fenestrale was initially collected from parasitized potato tuber moth larvae (PTM, *P. operculella*) infesting potato cultivation field in Jeju, Korea in May 2009 and has been maintained in the Highland Agriculture Research Center (HARC), Daegwallyeong, Pyeongchang, Gangwon, Korea. *D. fenestrale* was reared on PTM as a host in plastic cages (30 cm, cube shape) under the conditions of 25±2 ℃, 16 L : 8 D photoperiod, and 50-70% relative humidity. Third instar PTM larvae (5 days after hatch) were parasitized by *D. fenestrale* in an open-type cylindrical plastic cage (15 cm diameter, 30 cm height) for 24 h and parasitized hosts were reared in the same condition as the unparasitized larvae until emergence. The emerged *D. fenestrale* adults were collected everyday and allowed to mate for 24 h before use for parasitization. Adult wasps were fed with 10% sucrose solution.

2.1.2. Lepidopteran hosts

The PTM larvae were collected from Jeju, Korea, together with parasitic wasp, *D. fenestrale*. The emerged PTM adults were allowed to mate in an open-type cylindrical plastic cage (15 cm diameter, 30 cm height) with a filter paper on the top for oviposition. The PTM eggs attached to the filter paper was transferred to plastic cage (30 cm, cube shape) with potato tuber plant (*Solanum tuberosum*).

2. 2. Characterization of *Diadegma fenestrale* Ichnovirus (DfIV)

2. 2.1. Morphological characterization of DfIV

D. fenestrale females were dissected to observe the general morphology of the female reproductive organ. One-day old female wasps were anesthetized by ice and then, dissected in phosphate-buffered saline (PBS, 10 mM sodium phosphate, 150 mM NaCl, pH 7.2). The reproductive organ was observed under a Leica M205C stereomicroscope (Leica, Wetzlar, Germany) or Nikon Eclipse E600 microscope (Nikon, Tokyo, Japan) and photographed with a DFC450 or DFC420C camera system (Leica).

The ovary tissue was dissected in PBS from one-day old female wasp and fixed immediately for 2 h at room temperature in 0.2 M sodium phosphate buffer containing 3% glutaraldehyde. The tissue was post-fixed for 2 h in the same buffer containing 2% OsO_4 and exposed to 0.1% aqueous uranyl acetate overnight. Dehydration was performed with 30–100% ethyl alcohol in six sequential steps with each for 30 min. The dehydrated tissues were embedded in spur resin (EMS, Hatfield, PA, USA) and incubated at 70°C for 18 h. Ultra-thin (80 nm) sections were prepared on an ultramicrotome with a glass knife. Specimens were double-stained with 2% uranyl acetate and 0.5% lead citrate for 15 and 7 min, respectively. Localization of the viral particles in the ovary tissue and their morphology were examined with the transmission electron microscopy.

2.2.2. DfIV genomic DNA (gDNA) extraction

Ovaries were dissected in PBS from one-day old female wasp. The dissected ovary tissues were homogenized by glass-glass micro tissue grinder (Radnoti, Monrovia, CA, USA) and the homogenate was passed through a 0.45-μm syringe filter (MFS, Dublin, Ireland) and centrifuged for 30 min at 15,000 × *g* at 4°C. About 100 female adults were used for genomic DNA (gDNA) extraction. The pellet was resuspended in DNAzol (MRC, Cincinnati, OH, USA) and homogenized by a disposable tissue grinder. The resulting homogenate was centrifuged for 15 min at 12,000 × *g* at 4 °C and the supernatant was transferred to a new tube. The DfIV gDNA was precipitated by adding the same volume of ethanol and centrifuged for 10 min at 10,000 × *g* at 4 °C. The pellet was

washed with 75% ethanol, dried and then resuspended in nuclease free water. DfIV gDNA was quantified using a NanoDrop ND-100 spectrophotometer (NanoDrop technologies, Wilmington, DE, USA). To visualize the viral segment DNAs, 2 µg of DfIV gDNA was separated on 0.5% agarose gel at 30 V for 9 h.

2.2.3. DfIV genome sequencing

The whole DfIV genome shotgun sequencing was performed at Macrogen Inc. (Seoul, Korea) using the GS-FLX sequencer (Roche, Basel, Switzerland) with FLX-plus chemistry sets according to the GS-FLX manual. The adapter and primer sequences were removed and the DfIV genome was assembled using the GS de novo assembler (Newbler v 2.6, Roche). All the contigs obtained were analyzed using the Blast2GO and full length segments were amplified from DfIV gDNA using KOD-FX polymerase (TOYOBO, Osaka, Japan) with each contig primers (Table 1). PCR reactions (20 µl) contained 2 µl of DfIV gDNA (20 ng) were subjected to cycling conditions of 3 min at 94 °C followed by 35 cycles of 94 °C for 20 s, 55 °C for 20 s and 68 °C for 1 min with a 3-min final extension. PCR products were purified by Wizard SV gel and PCR clean-up system (Promega, Madison, WI, USA), and directly sequenced by cycle sequencing or cloned into pGEM-T Easy vector (Promega), then followed by sequencing (Macrogen). Open reading frame (ORF) was predicted by ORF finder program (NCBI). Functional gene prediction was performed using DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST) and cluster analysis was done by cluster W method using Lasergene (DNASTAR, Madison, WI, USA) with pfam database (Punta et al., 2012; Sonnhammer et al., 1997). Amino acid sequence alignment and phylogenic tree construction for *reps*, *cys-motifs*, *vankyrins* and *vinnexins* were conducted using Lasergene (DNASTAR) and MEGA 5.2 (Tamura et al., 2011).

Table 1. Primer used for the full length sequencing of DfIV genome segments

Target	Forward	Reverse
DfIV contig 1	CGACAGATCGCTGTGCCAA	GAGGTCACTCAAGTGCCATCTT
DfIV contig 2	GGCATAGCGATAGCTGGAAC	GGGAAGGACGAAATCGAGTC
DfIV contig 3	GTTGCGTTCTGGAACACTCAA	CGGCAAGCTCATGGACCAT
DfIV contig 4	CCGCGTTCCTGCAGCTTAA	CCTGGGTGACACAGTGACAT
DfIV contig 5	CCGTTGGTTGTCATAGCTAACTG	CGAGCGTCGAGCAACAAATATT
DfIV contig 6	CGTCGGAAGAGTGTGGGTATA	CCTGAGCGGCCCTCAGTT
DfIV contig 7	GGCATGGCACGCATTAGAATG	CACGACGCAGCTCCATGTA
DfIV contig 8	CTCGGACGGTACAATGGTTG	CCAGTGGTCATCGTGACATTGT
DfIV contig 9	CTGTGCAGAAGAGCAGCAAAAAC	CAGCTGCACAGGAATTACAGGAA
DfIV contig 10	GGTACGGTCGAATACGTTCAAA	GTGGTACGGTCGAATACGTTc
DfIV contig 11	GGCAAGAGGCGAATTGACA	CCGACTGAGTGTTGTAGGTGT
	GTCCGCCGCGTCAATATTTATAG	GCATGGCCCCACCAAGTAT
DfIV contig 12	GCTCAGCCAGACCGCAAA	GCGCAAGCAGCGGAGATA
DfIV contig 13	GTGGCGGTATTTGCACGTATC	GCGACACGAGCTGAATCAACA
DfIV contig 14	GCGCAGTCACGCTCATCAT	GCTACGCTGGAGGTTCAAGA
DfIV contig 15	GAGCACTGGAGCTGACTCTT	CCCAACCAAGTACTGACCGAA
DfIV contig 16	GGCGTTTGCTCTGGATGTT	GCGCTCGAACCTCTTTCCTAA
DfIV contig 17	GGACTGCAGCGCAGCATT	CACAGAGTTGTCATGAGGGAAAC
DfIV contig 18	GGGCCTTGCTAATTCGCAAA	GGAGCCTGGCTCATGACTAA
DfIV contig 19	GGGTTCTCCCCCTAGACAAA	CCCCTGGAAGACATAGGTTGTTAT
	GCCGTGAGCAGCAATGAATG	GACGCCATGCTAACGGACA
DfIV contig 20	CCGTCCGAAGTTAGGAAGCTTT	GGTCCGCATATTTCTTGCTGAAA
DfIV contig 21	GAGTCGTCCCACCAGGTAT	CCGCACTCTTGCAGGGAAA
DfIV contig 22	CGCACCGGCATTTCGTTCTATA	CGCCGATACTCATATCTGGCTTG
DfIV contig 23	GCGTGGTATTTAAGCACATCACA	GGAGTACAGGCGTGAGGTATA
DfIV contig 24	CCGCTATCCCGTCCTTCAAT	GATAGCTGGACCACCGCAAA
DfIV contig 25	GGTCGAAGGTTTATGACCTAATCTG	GCCAGGCCGTCATACTAGAAA
DfIV contig 26	CAAAGTGGGTGCTAGCGTTA	CCACCGGTTCTTAAAGTTCGAT
DfIV contig 27	GAGACTGCAGGCTATGCAAA	GCGTTATAGGGTGTCCAGACTAA
DfIV contig 28	CGAGCGCGGACAAGTTGAA	GCTTGCTGCCCTTGTCACTT

Table 1 (continued)

DfIV contig 29	GGCCACGGAATCTCTACAGATT	CCAACCTCACCGACATCTTTCAA
	CGAGACACGCGCCTCATAT	CGCTGGGATCCCAACACTA
DfIV contig 30	GCATGCTGGCTTGCACTT	CACGCCGCCATCACAACAA
DfIV contig 31	CTGCGATAAGCAAGCGAGTT	GTCGATGCGAAACCTGGACA
DfIV contig 32	CGACAGAGAGAGCGATGCTAT	GTATGTCAGCGTTGGGATGTGAT
DfIV contig 33	GCGGATGGTTGTCTTCGTAAGT	CAGCCGACTTGTGACGTACT
DfIV contig 34	CCGGAGGGAACAGTATGTTCT	CAAGAGTTCCATACGTTTCGCAGA
DfIV contig 35	CGAGTGTCCGCATGAGGTTT	CACCACAGCGGCAGATATGTT
DfIV contig 36	GGCTTGTCACCATGCTGTATA	CACACGCCGCTATCACAACAA
	GCGGTCTGAAATGGCTGAATAAAC	CACCAAGCTCCCAACTGCTAA
DfIV contig 37	CGGTGATTGTTCTTCTGCTGTTT	CTGGGGGAACCCTGTCTTT
DfIV contig 38	GGGATGCATTTGCCTCAGAAT	CGTCCGCCATCAGAACCAAA
	GGCTCAAGCCGCTGTTGATA	GGGTTTCAGAGCTGCGCATAA
DfIV contig 39	GGCGGCTCCTGACATTGTAT	CCAACCTCACCGACATCTTTCA
DfIV contig 40	CGCCGTCTTAATGACCGCTTA	CCCTCATTGTTGCGAGTGATG
DfIV contig 41	GATAGGTCGGGTGCGTCAT	CAGCTGGAGATTCAATACACGTTC
DfIV contig 42	GCGTCATGCGAGCCAAGTAT	GCAGCATCGTCTATTCGGAGTAT
	CCTGCATCGCTTTCGTATACAGT	CACACCCGTGCATGGTAGAT
DfIV contig 43	CAGGTGCCTATTCAACAGCATc	CGTCCAAGTCGAACACCTTCAA
DfIV contig 44	CCCCACTACAGATCGAGTACAT	CCTCTCACCTATCTCTCGGAGAA
DfIV contig 45	GCTGCGAGGGAGTCTCATA	CTGTCCGACAACGTTGAGAAAG
	GGCCGGGGTTTGAGTTGTAT	CGCCGGTCATTCTCTACTTGAA
DfIV contig 46	GGGGACGCGTTCAAGAAACT	CGACCGCATGACGATCGATA
DfIV contig 47	CCTGATGCGTTTCCAGAATCAGT	GCACTGCCGAATTCTGACAAT
DfIV contig 48	GGCACGGCAACTCTGAAATAC	CGACTTGTCCTCTTCTATGCTCTT
DfIV contig 49	CCGTTCTTGAGCGAAGAGTGTAT	CAGTCAGCTCTACGTGCTATGTTT
	CCTCCGTAGCATTCTGCACAAA	GAGCGAGTGTCGTGGCAAAA
DfIV contig 50	GCACGGTCGTGACTTCAGTTA	CAGATCGGAGTCCGTCACA
	GCTCTGCGTGCCTACCATTTA	CGGCGTGAGAGACGAACTTTT

Table 1 (continued)

DfIV contig 51	GGCTATGACGTCCATCGATCA	CCAGGAAATCTCTTGTGAGATCAC
DfIV contig 52	GGCGGAGGTGTTGCTGAAA	GCATCGTGTCAGAGACACACATA
DfIV contig 53	CTGCTGACCTCATGCCTGATA	CTGGCTTACAGGGAGCTCATA
	GCGCAAGGAAGCGATAACGTAT	GTCTACCCAGGTAAGCTGATTGT
DfIV contig 54	GACTGGGCGGCTATAAGTGTTG	GGCAGGATGCGTATCGAGAT
DfIV contig 55	GCCAATGGATTCAGGTTCCAAg	GTGGTGCAGCGTGATACAGAAA
DfIV contig 56	GGCCTGCTAACAGAATCCTGTAT	GCGCAAGGGCATGTGGATAAT
DfIV contig 57	CCGGTCAGATCTATCTTCGGTAT	CACGTGTGTCGCGGTAACAAT
	GCTGGGCATCGTCGATGTT	
DfIV contig 58	CGAGCTGACTTCACCGTTCTT	CTGAGACGGTCGAACGACTA
DfIV contig 59	CCACAGGTGTAGCCATGCTA	CGTCGGGTTACAGAAACTCTAC
DfIV contig 60	GTGCAGTGCATTCGGCAAT	CAGACAGGCGAGGTGTCTA
DfIV contig 62	CGACGTCGCTATTTGCAGTCT	GTTCACCACATGACCACACTGATA
DfIV contig 69	GAGGGCTTTGTCGGCTCTAA	GCCAGTATGCTTCGATCAGGTT
DfIV contig 70	CGGCAGGGCGTTTACTGATTA	GCCAGATGCTGCATGTCCAT
		CTGCCTGTTCGCATCTCTCTTA
DfIV contig 78	GCACTGTCCGTTACAGCTTTG	GCTACCACGTCATCCCATGT
DfIV contig 94	GGCGTTCGCCACATAACTACA	CCCGCATAACCTGACGAATG
DfIV contig 97	GCTCCCTAGCTCGCCAATA	CACACAGGGTCTTGTTGCTACA
DfIV contig 104	CGTCCAACACACCGAGATCTT	GCACATCGACTGATTCTCGAAAC
DfIV contig 113	CGGACCCGATTGTGATACAGA	CGACCCATCTGTGAGGGAAT

3. Results

3.1. Characterization of *Diadegma fenestrale* Ichnovirus (DfIV)

3.1.1. Morphological characterization of DfIV

The DfIV was discovered in the female reproductive organ, particularly in the calyx tissue of *D. fenestrale* as other PDVs (Wyler and Lanzrein, 2003). When the ovary was observed under stereomicroscope, blue color was detected in the oviduct (Fig. 2). To confirm the DfIV existence in ovary, ultra thin cross sections of the distal ovary, proximal ovary and calyx regions were prepared and examined using TEM (Figs. 3-5). The stem cells, early immature oocytes, were observed in the distal ovarial region, but no viral particles were observed (Fig. 3). Single ovary was composed about 10 ovarioles and more than six ovarioles showed in Fig. 3B and about five oocytes located in each ovariole (Fig. 3C). Double membrane nucleus was observed inside of oocyte (Fig. 3D). Five more developed oocytes were observed (Fig. 4B) and surrounded by follicular epithelium (Figs. 4C, D). Oocytes were at vitellogenic development. Mature oocytes were inside of ovarian epithelium in calyx with viral particles (Figs. 5B, C). This virus was named as *D. fenestrale* Ichnovirus (DfIV). DfIV exhibited the typical double membrane IV shape (Figs. 5E, F) (Webb, 1998.). DfIV was only observed with the mature oocyte while virogenic stroma was detected in the ovarian epithelium (Fig. 5D). These results suggest that DfIV was replicated in the ovarian calyx epithelium and, like other PDVs, concentrated inside the calyx and lateral oviduct with mature oocyte (egg) (Bae and Kim, 2004; Burke and Strand, 2012; Huang et al., 2008; Webb, 1998.).

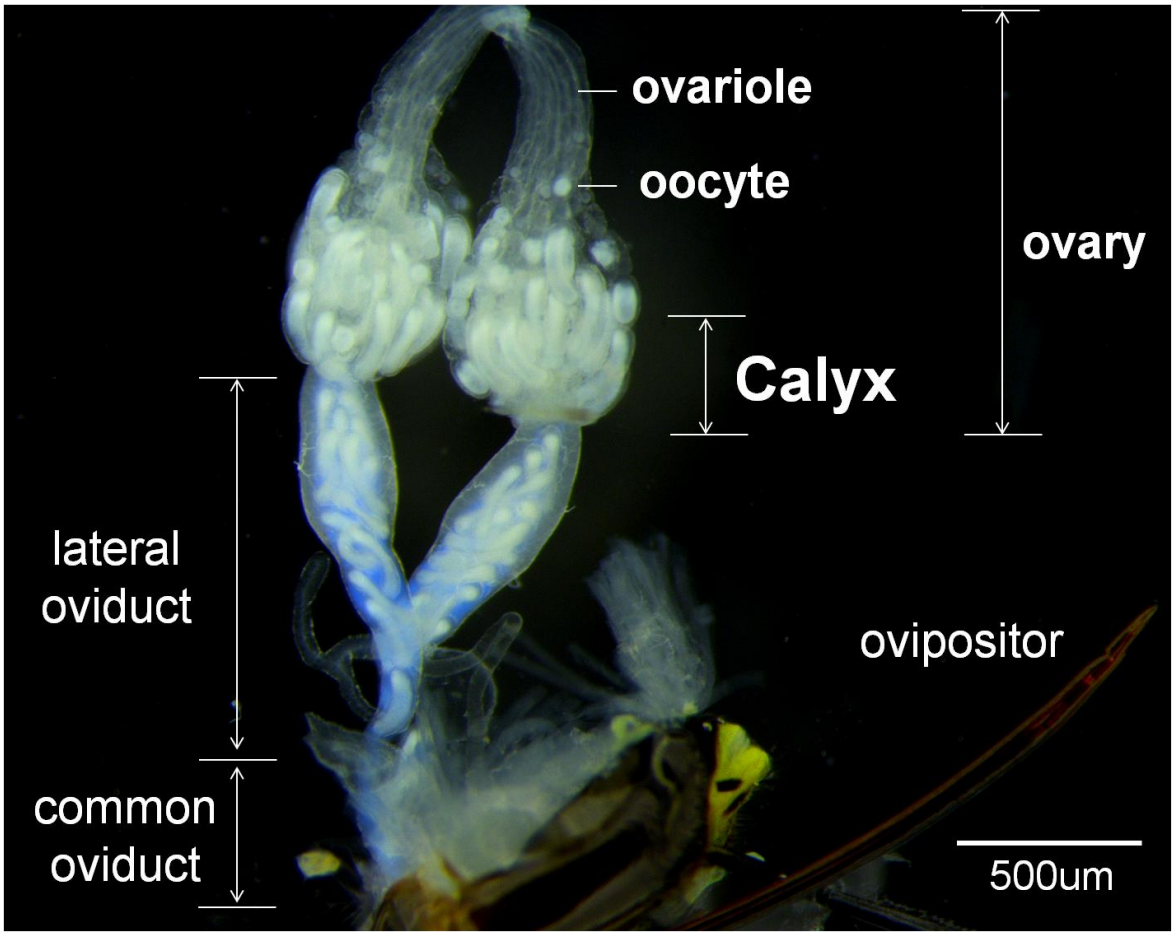


Fig. 2. Representative female adult's reproductive organ structure for *D. fenestrale*. *D. fenestrale* female adult was collected at one day after emergence and anesthetized using ice. After being dissected from the female abdomen in PBS, the reproductive organ was photographed using a stereomicroscope.

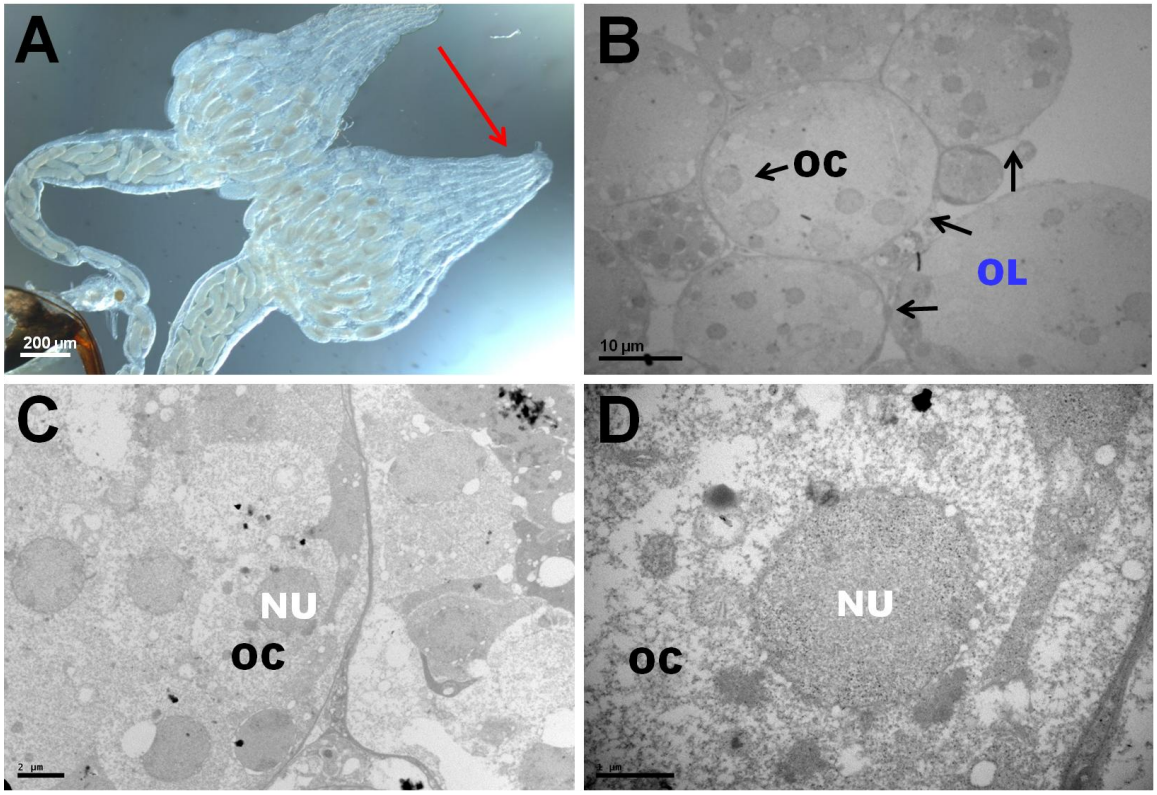


Fig. 3. Oogenesis at the germarium of *D. fenestrale* ovary. Single ovary was composed about 10 ovarioles. After being dissected from the female abdomen in PBS, the ovary was photographed using an optical microscope (A) and transmission electron microscopy (TEM; B-D). A red arrow indicates the cutting site and direction of the cut (A). More than five immature oocytes (OC) were located in each ovariole (OL) (B). A detailed view of the ovariole is shown in C. Five oocytes and their nucleus (NU) observed in an ovariole. More detailed view of oocyte has been presented in D. Red triangles indicate oocyte (B-D).

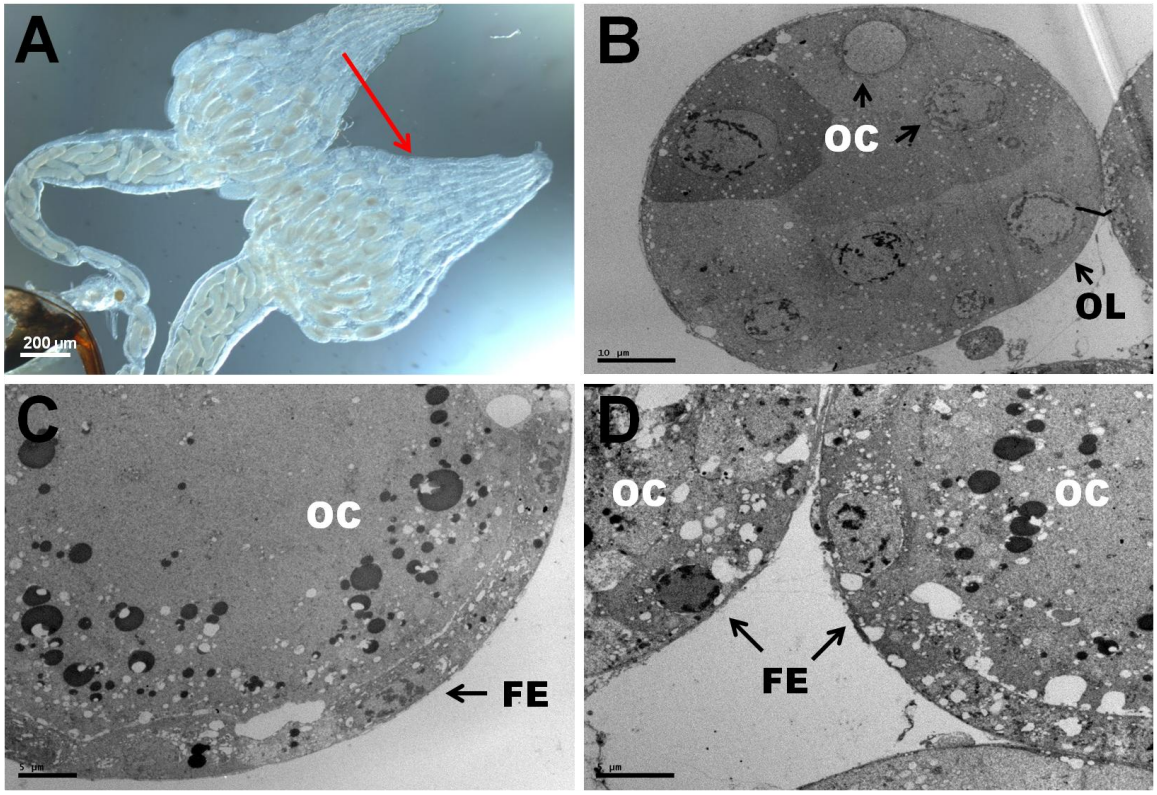


Fig. 4**.** Oocyte (OC) is surrounded by follicular epithelium (FE). OCs are in vitellogenesis. After being dissected from the female abdomen in PBS, the ovary was photographed using an optical microscope (A) and transmission electron microscopy (TEM; B-D). Red arrows represent the cutting site and direction of the cut (A). Six OCs were located in an ovariole (OL) (B). A detailed view of the OC is shown in C and D.

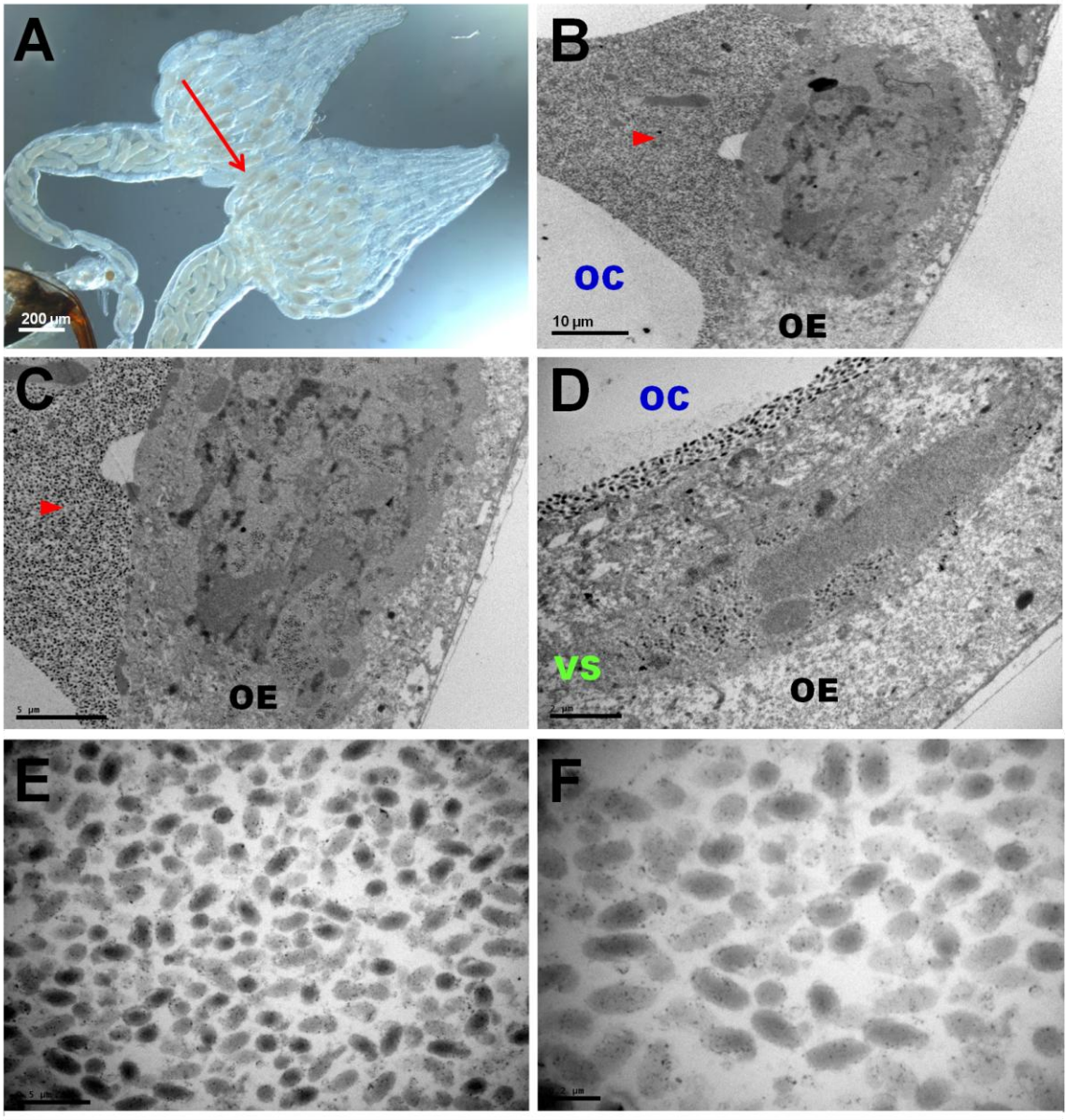


Fig. 5. After vitellogenesis, Oocyte (OC) surrounded by virion particles at the calyx area. Ovarian epithelium (OE) contains virogenic stroma (VS) and release the virion particle to the calyx chamber. Red triangles indicate the presence of DfIV particles. DfIVs were located within the OE with OC (B-C). DfIV showed typical double membrane envelope (E, F). A red arrow indicates the cutting site and direction of the cut (A).

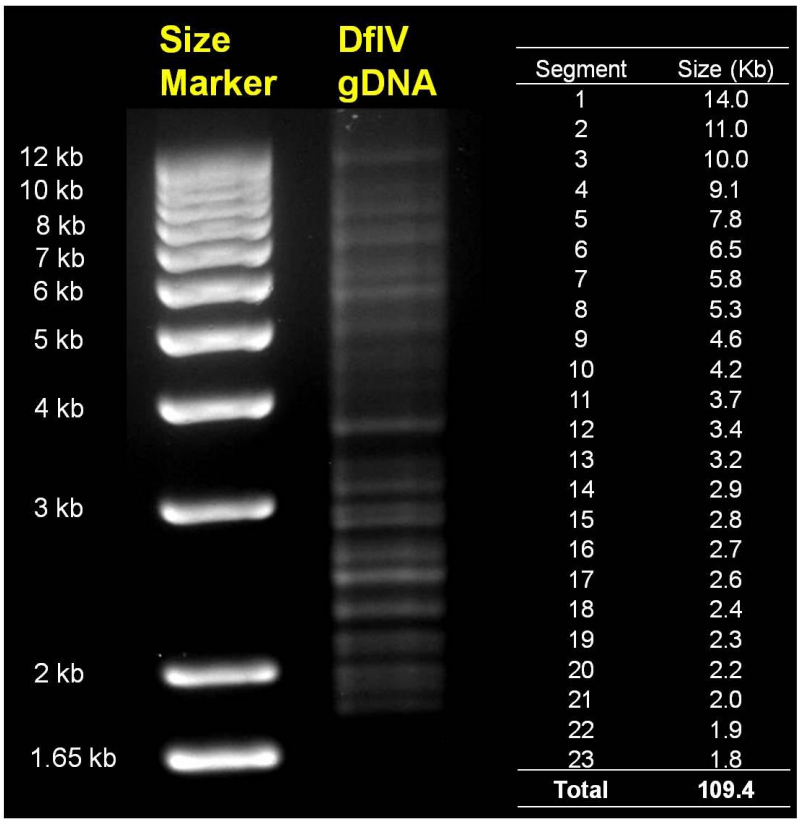
3.2.2. DfIV genome annotation

The DfIV genome size was estimated by gDNA gel electrophoresis (Fig. 6). Twenty three segments were visible and their apparent total size was estimated to be approximately 110 kb. The DfIV genome draft was constructed using GS-FLX plus. A total of 20,810,524 bp was sequenced through 51,684 reads and assembled to about 120 contigs. Subsequent primer walking PCRs based on the NGS sequences indicated that the total DfIV genome was 247,191 bp and composed of 65 segments. Among the 65 segments, BLAST analysis showed that 63 segments were similar to HfIV and an average GC contents was 43.3% (supplementary table 1). The underestimation of genome size analyzed by gel electrophoresis may be due to poor separation of supercoiled genome segments and difference in genome segment abundance. Genome size, GC content, number of segments and genes from DfIV were compared with those of other PDVs. Genome size and GC content were highly similar among DfIV, HfIV and CsIV. *D. fenestrale, H. fugitivus* and *C. sonorensis* are members of the same subfamily, Campopleginae. However, the number of segments and genes were variable (24 to 65 and 105 to 135) and the degree of genome segmentation was higher than assessments for both HfIV and CsIV. Some segments were partially overlapped due to the intramolecular recombination of larger genome segments (Kroemer and Webb, 2004). Its genome segments size were ranged in 1.426 kb to 6.602 kb. The median size was 3.769 kb. These genome characters are much different to those of GfIV, CcBV, MdBV and CvBV (Table 2).

The initial criterion for predicting DfIV ORFs had a minimum size at 201 bp (67 amino acid codons). 377 ORFs were predicted from these 65 genome segments and some ORFs located in different genome segments (supplementary table 2). DfIV genome segments and their annotation results showed in Fig. 7. Meanwhile, 99 genes were predicted using DELTA-BLAST with cluster analysis using the cluster W method. Most of these genes were matched with that of other IVs such as CcIV, CsIV, GfIV, HdIV, HfIV, and TrIV. The repeat element protein gene *rep* was mainly present among these genes containing functional domains. 40 *rep*, 12 cys-motif gene; *cys-motif*, 8

viral ankyrin gene; *vankyrin* and 6 viral innexin gene; *vinnexin* families were comprised of over 60% among the all genes (Fig. 8A). With these gene families, 2 polar residue rich and 1 N-gene were also found as other reported IVs (Fig. 8B). Generally *rep* was the most abundant gene family in IVs and DfIV had the high number of *rep*. Other genes were variable in numbers and proportions.

DfIV genome segments were aligned and analyzed in their phylogenetic relationship (Fig. 9). Following the maxium likelihood phylogeny of DfIV genome segments, six main gene families *(rep, cys-motif, vankyrin, vinnexin,* polar residue rich and N-gene) were revealed in the encoded segments. Forty DfIV *reps* were located in 48 loci from 25 segments, twelve *cys-motifs* were encoded in 12 loci from 7 segments, eight *vankyrin* were located in 9 loci from 5 segments, six *vinnexin* were located in 10 loci from 10 segments and two polar residue rich located in 2 loci from 2 segments. First divergent point, some *rep* contained segments were grouped and also generally *rep, cys-motif, vankyrin* and *vinnexin* contained segments were grouped (Fig. 9). *Reps* and *cys-motifs* contained segments were grouped two subsets likewise that of gene sequences. From this result, DfIV genome segments recombination and some gene duplication could be predicted. Relative segment abundance of DfIV was predicted by number of reads from GS-FLX data (Fig. 10). The abundance of the least abundant segment, DfIV S-57, was standardized to a level of 1. The abundance was really varied and DfIV S-17 was the most abundant. Any correlation was not found between DfIV genome segments' copy numbers and their phylogenetic relationship. On the other hand, some correlation was found found between DfIV genome segments phylogenetic relationship and that of genes, particually *reps*.



Segment	Size (Kb)
1	14.0
2	11.0
3	10.0
4	9.1
5	7.8
6	6.5
7	5.8
8	5.3
9	4.6
10	4.2
11	3.7
12	3.4
13	3.2
14	2.9
15	2.8
16	2.7
17	2.6
18	2.4
19	2.3
20	2.2
21	2.0
22	1.9
23	1.8
Total	**109.4**

Fig. 6. Genome segment structure was visualized using EtBr staining following gel electrophoresis of DfIV. The DfIV gDNA (2μg) was separated on 0.5% agarose gel at 30V for 9 hours. Twenty three segments were identified and their genome sizes were estimated (right table). By adding all segment sizes, total DfIV size was estimated to be about 110 kb.

Table 2. Genome size, GC%, number of segments and gene comparisons for genome identified polydnavirus with DfIV

Organism	BioProject [a]	Size (Kb)	GC%	No. of segments	No. of genes
Diadegma fenestrale ichnovirus **DfIV**		247	43	65	99
Hyposoter fugitivus ichnovirus **HfIV**	PRJNA18779	246	43	56	135
Campoletis sonorensis ichnovirus **CsIV**	PRJNA16738	247	41	24	106 [b]
Tranosema rostrale ichnovirus **TrIV** [c]		250	42	40	86
Glypta fumiferanae ichnovirus **GfIV**	PRJNA18767	292	37	105	103
Cotesia congregata bracovirus **CcBV**	PRJNA14556	568	34	30	182
Microplitis demolitor bracovirus **MdBV**	PRJNA15245	185	34	15	60
Cotesia vestalis bracovirus **CvBV** [d]		540	35	35	157

[a] BioProject numbers and polydnavirus genome information cited from NCBI homepage (http://www.ncbi.nlm.nih.gov/genome).

[b] CsIV gene number edited from 5, based on reference (Tanaka et al., 2007)

[c] TrIV genome do not completely sequenced genome size and number of segments were predicted (Tanaka et al., 2007)

[d] CvBV genome reported at 2011 and 2009 as CpBV, *Cotesia plutellae* bracovirus (Chen et al., 2011; Choi et al., 2009c). *C. vestalis* and *C. plutellae* were identified same species.

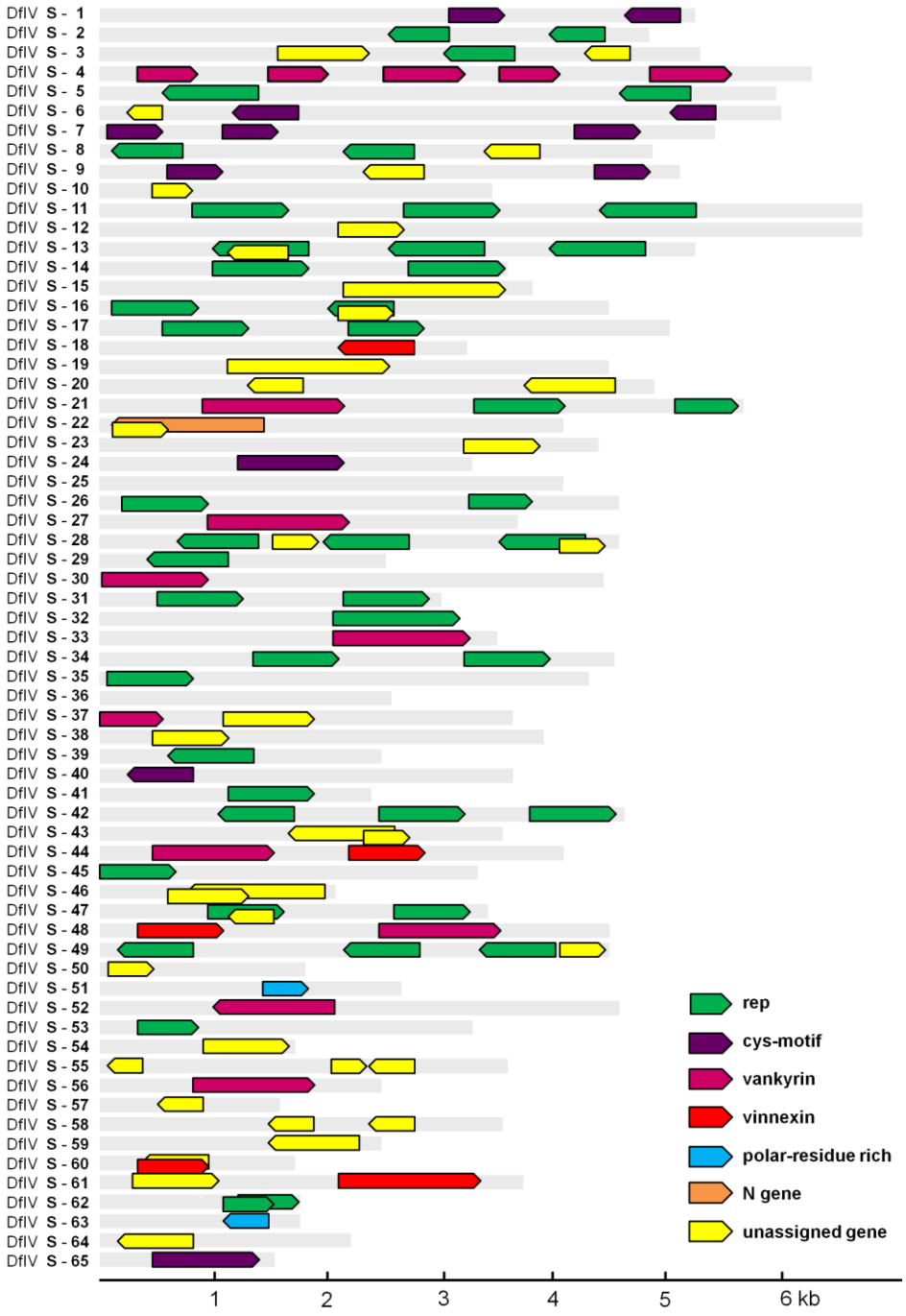


Fig. 7. Graphical representation of DfIV genome and its annotated genes, with 65 non redudndant circular genome segments shown as linear molecules. DfIV genome segments ranged from 1,426 to 6,654 bp. Colored box showed the sizes and locations of gene families with directions indicated by the arrowhead on each box. Gray regions represent non-coding DNA.

A

Genes	No. of genes
rep	40
cys-motif	12
vankyrin	8
vinnexin	6
polar residue rich	2
N-gene	1
unassigned gene	30
Total	**99**

B

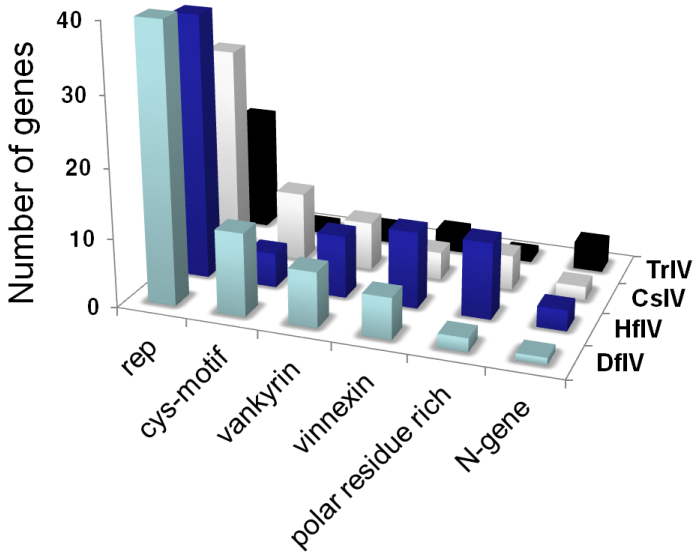


Fig. 8. DfIV ORFs were predicted by ORF finder from DfIV genome segments and 99 genes were confirmed by DELTA-BLAST with cluster analysis using the cluster W method (A). The bar graph shows six gene families composition ratio from DfIV with other IVs such HfIV, CsIV and TrIV (B). DfIV had 99 functional genes. 69 genes were assigned to six gene families while 30 genes were unassigned. HfIV, CsIV and TrIV had a total of 150, 106 and 86 genes with 73, 48 and 51 (with 7 TrIV genes) unassigned genes, respectively. Unassigned genes do not show.

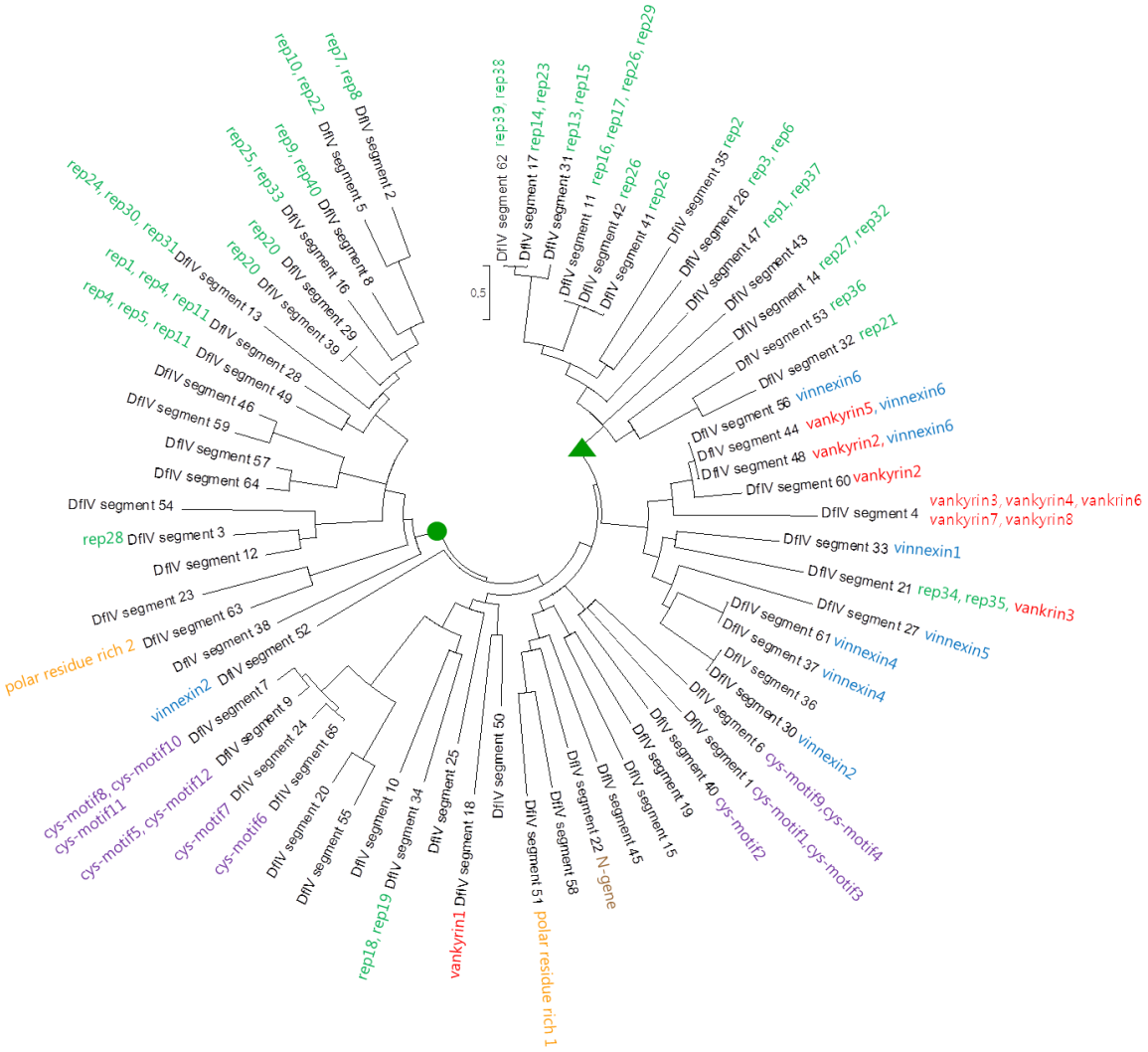


Fig. 9. Maximum likelihood phylogeny of DfIV genome segments and six main gene families (*rep*, *cys-motif*, *vankyrin*, *vinnexin*, *polar residue rich* and N-gene) were revealed in the encoded segments. Forty DfIV *reps* were located in 48 loci from 25 segments, twelve *cys-motifs* were encoded in 12 loci from 7 segments, eight *vankyrins* were located in 9 loci from 5 segments, six *vinnexins* were located in 10 loci from 10 segments and two *polar residue richs* located in 2 loci from 2 segments. Green circle represents first divergent point and green triangle indicated *rep* contained segments group, except segment 43. The tree was produced using MEGA 5.2 using the cluster W method. The scale indicates the percentage of divergence.

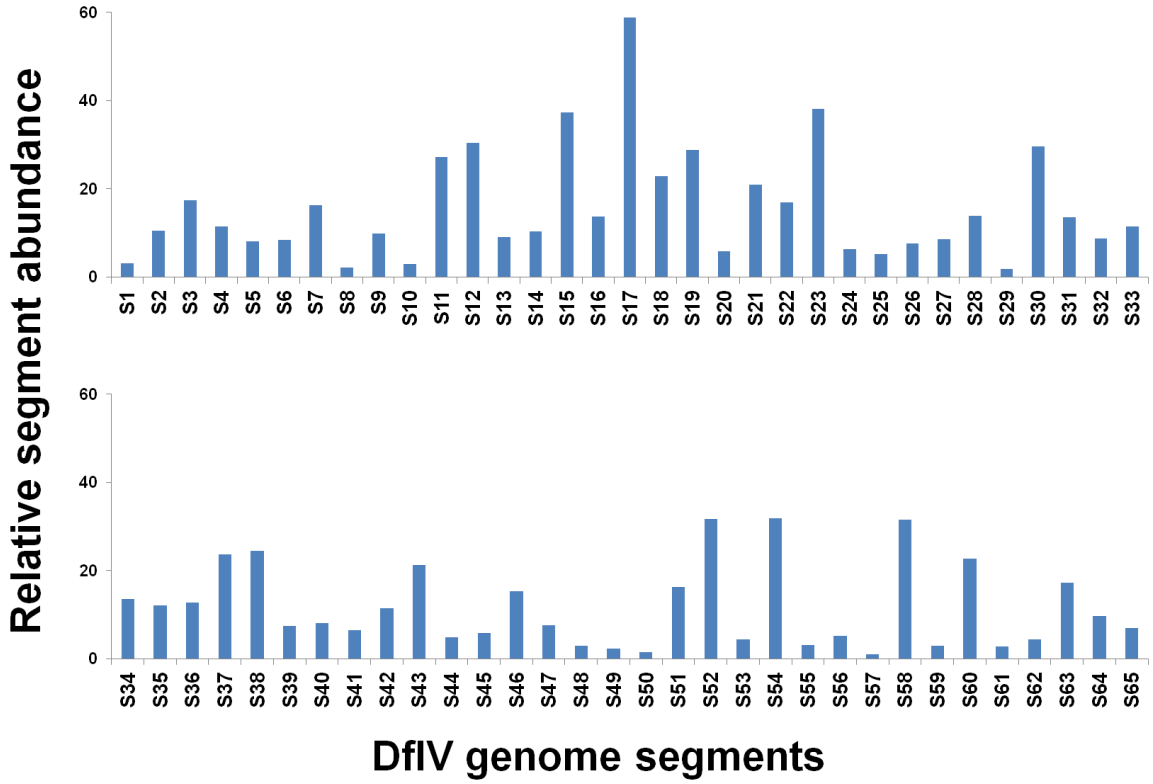


Fig. 10. Relative segment abundance of DfIV in extracted genomic DNA. The abundance of the least abundant segment, DfIV S-57, was normalized to a level of 1. The relative abundance of each DfIV segment is the normalized results for the GS-FLX read counts of each segments. DfIV S-17 was the most abundant.

3.2.3. Phylogenetic analysis of DfIV genes

IVs generally encode *rep*, *cys-motif*, *vankyrin* and *vinnexin* genes, in which BVs do only vankyrin (Choi et al., 2009c; Clavijo et al., 2011; Cui et al., 1997; Cui and Webb, 1996; Espagne et al., 2004; Hilgarth and Webb, 2002; Kroemer and Webb, 2004; Rasoolizadeh et al., 2009; Tian et al., 2007). These genes are important for parasitoid survival in lepidopteran host (Webb, 1998.). To better understand DfIV genetic characteristics and relationships among the PDV, four gene families were used in phylogenetic analysis.

Rep was the most abundant and diverged gene family in DfIV. Their gene sizes varied from 240 to 861 bp for *rep* (average length was 662 bp). Although the function of *rep* has not yet been fully elucidated, their conservation among IVs and abundance in viral genomes both suggest that they play an important role in viral maintenance (Galibert et al., 2006). Forty *reps* were found and these were diverse in terms of sequence and length (Fig. 11). Most *reps* showed about 200 amino acid lengths, but *rep* 4, 26, 36 and 37 were shorten in 3' region. Even though, *rep* 38 and 39 do not well aliened in Fig 11, highly matched to that of HfIV in DELTA BLAST (supplementary table 2). *Reps* were only observed in IV, such as HfIV, CsIV and TrIV (Galibert et al., 2006; Hilgarth and Webb, 2002; Rasoolizadeh et al., 2009), and DfIV *reps* were highly differentiated in each of the IVs (Fig. 12).

DfIV *cys-motif* size were ranged 267 to 867 bp (average length was 496 bp) and that gene function was known as inhibition of the host's cellular immune system in CsIV (Li and Webb, 1994). However most functional analysis performed close related gene, *cys-rich*, particularly CsIV Vhv1.1. (Einerwold et al., 2001). Because of these reasons, DfIV *cys-motifs* aligned with *cys-motif* conserved domain and CsIV VHv1.1 homology domain (Fig. 13). Following the alignment, DfIV *cys-motifs* analyzed their phylogenetic relationship (Fig. 14). DfIV *cys-motifs* were grouped with other that of IVs such as HfIV and TrIV. *Cys-richs* were also separately grouped with that of IVs and some BVs. Alignment result confirmed that DfIV *cys-motifs* were more similar with *cys-motif*

conserved domain than CsIV VHv1.1 homology domain.

Vankyrin is known as lepidopteran host's transcription factor inhibitor (Kroemer and Webb, 2005) due to its homology to Iκβ (Kroemer and Webb, 2005). The Iκβ has ANK (ankyrin) repeat and this domain well identified in *Drosophila melanogaster* Iκβ, Dmcactus (Geisler et al., 1992). DfIV *vankyrins* were aligned with Dmcactus (Fig. 15) and analyzed their phylogenetic relationship (Fig. 16). Dmcactus has also six conserved domain ANK 1 to 6, but only four ANK domains (3 to 6) were predicted from the alignment result with that of other PDV's homology domain comparison (Lapointe et al., 2007). Eight DfIV *vankyrin* lengths ranged from 501 to 582 bp and their *vankyrins* were divided into three groups based on their origin, IV, BV or IV (only GfIV) and BV. Among the IVs, vankyrins were subsequently grouped three subsets. DfIV vankyrins were located 1, 3 and others in each subset, respectively

Vinnexin is a member of proteins that create gap junctions in invertebrates (Marziano et al., 2011; Phelan et al., 1998). In DfIV, six *vinnexin* lengths ranged from 1059 to 1194 bp, average was 1111 bp. DfIV *vinnexins* were aligned with innexin and showed the conserved domain (Fig. 17). However, relatively low similarities (29.3~48.7%) were observed. However, DfIV *vinnexins* were grouped with other that of IVs, viral innexin (Fig. 18). Insect innexins were mainly grouped four families, (innexin 3 and 7), (innexin 4, 5 and 6), innexin 2 and (innexin 1 and shaking B). However, *vinnexins were not* grouped with the insect innexins.

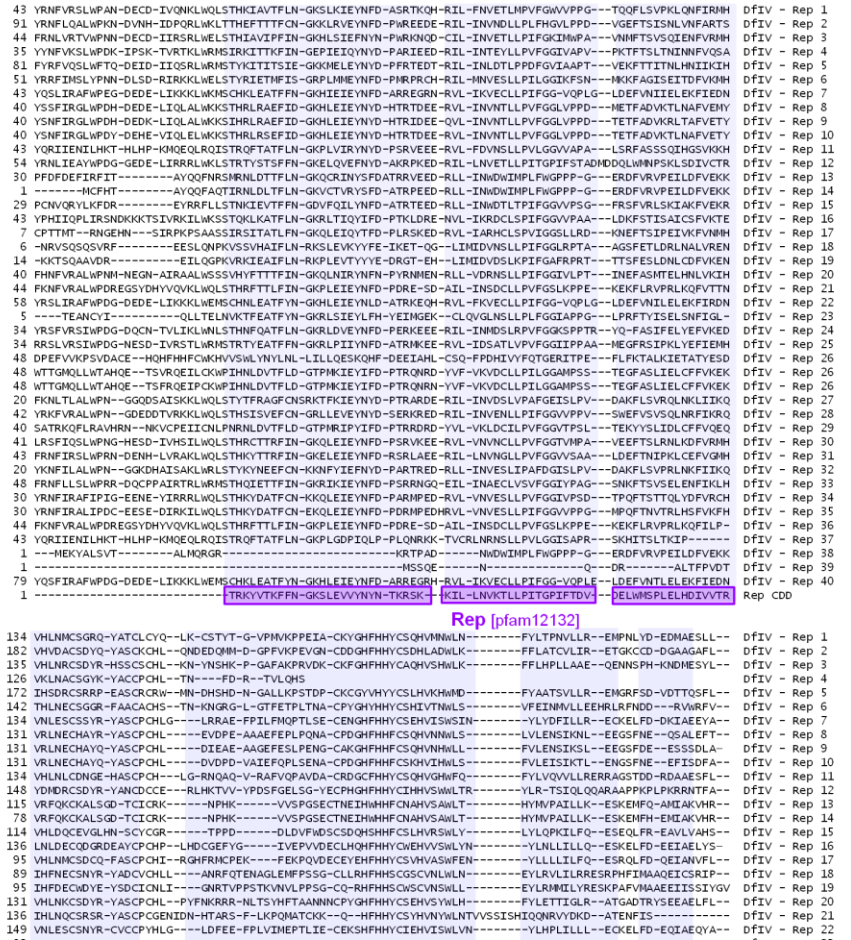


Fig. 11. Partial amino acid sequence alignment of DfIV *reps* using the clustal W method with the domain structure proofed *rep* in the pfam database (pfam12132). Conserved *rep* domains were highlighted in blue and purple. *Rep* domains were predicted by DELTA BLAST and the conserved domains program (http://www.ncbi.nlm.nih.gov/Structure/cdd).

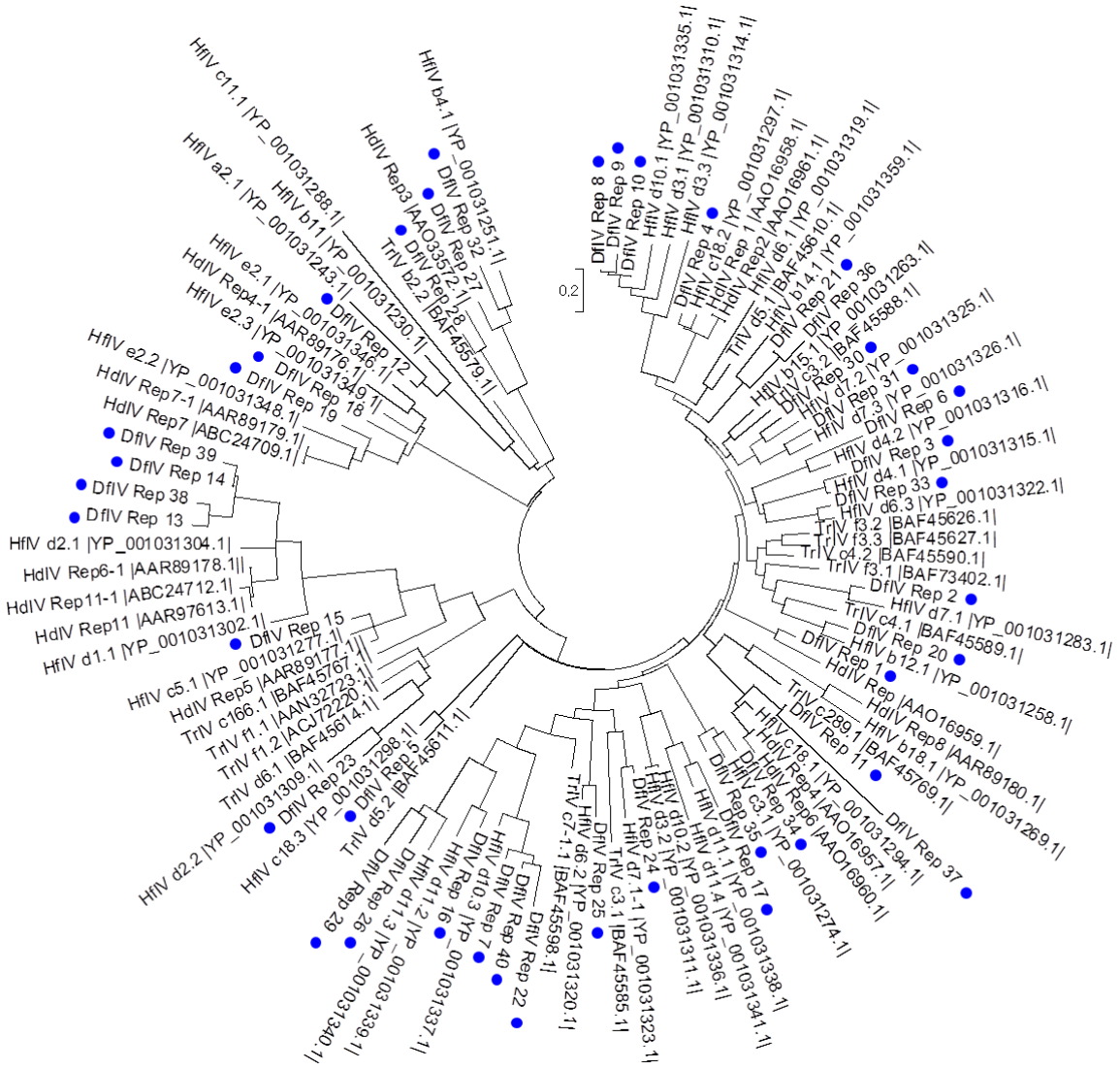


Fig. 12. Maximum likelihood phylogeny based on the deduced amino acid sequences of DfIV *rep*s (represented by blue dots) along with *reps* from other IVs such as HfIV, HdIV and TrIV. The tree was produced using MEGA 5.2 using the cluster W method. The scale indicates the percentage of divergence.

Fig. 13. Amino acid sequence alignment of DfIV *cys-motifs* using the clustal W method with domain structure proofed *cys-motif* in the pfam database and *cys-rich* from CsIV-VHv 1.1 (1XJ1_A) (Einerwold et al., 2001). The conserved *cys-motif* domains were highlighted in blue and purple. *Cys-motif* domains were predicted by DELTA BLAST and the conserved domains program (http://www.ncbi.nlm.nih.gov/Structure/cdd).

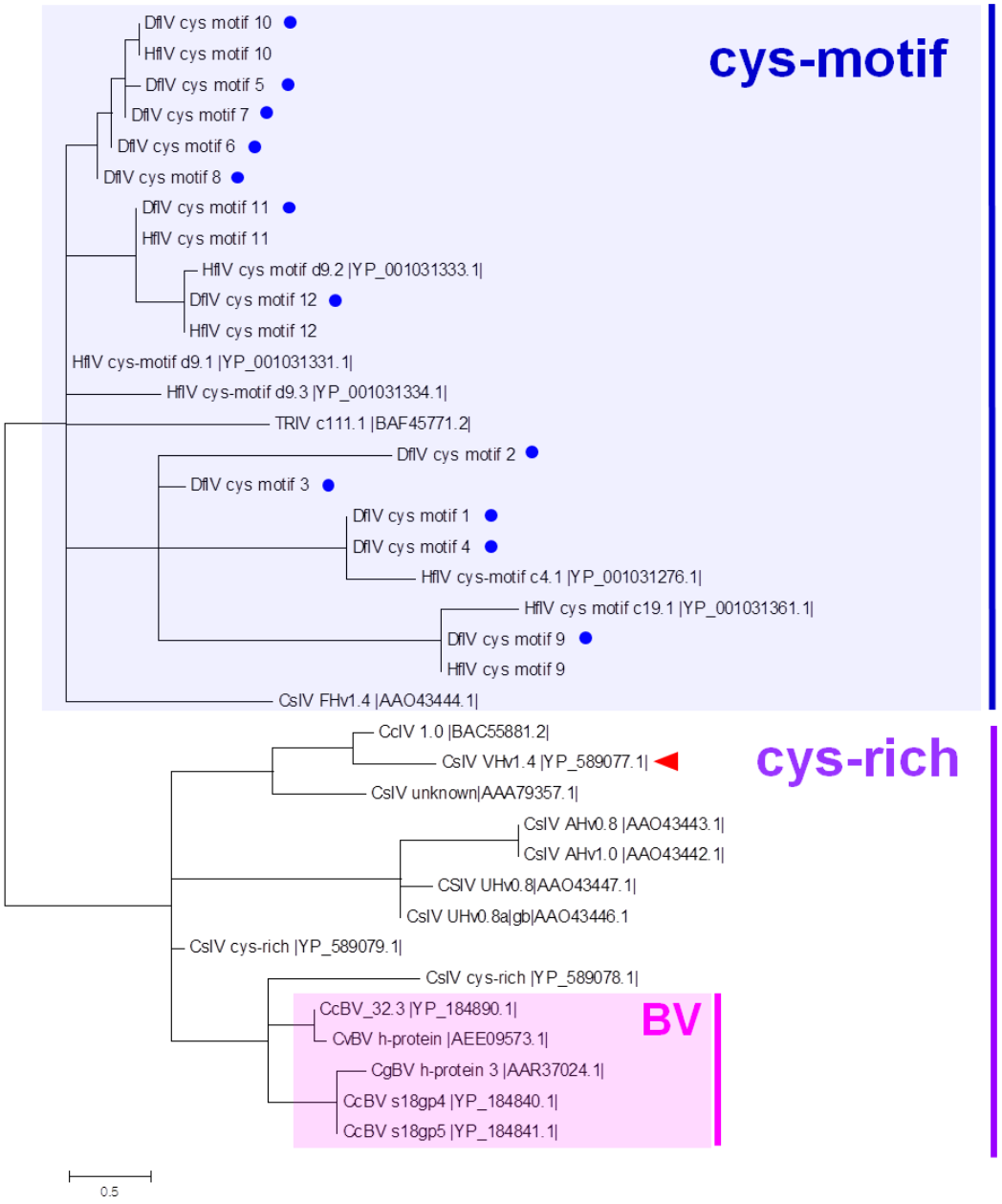


Fig. 14. Maximum likelihood phylogeny based on the deduced amino acid sequences of DfIV *cys-motifs* (pointed by blue dot) along with other *cys-motifs* and *cys-rich* from other polydnavirus. Blue bar and purple bar indicate *cys-motif* orthologous and *cys-rich* orthologous genes, respectively. Pink boxes and bars signify BV orthologous *cys-richs*. The tree was produced using MEGA 5.2 by the cluster W method. The scale indicates percentage of divergence.

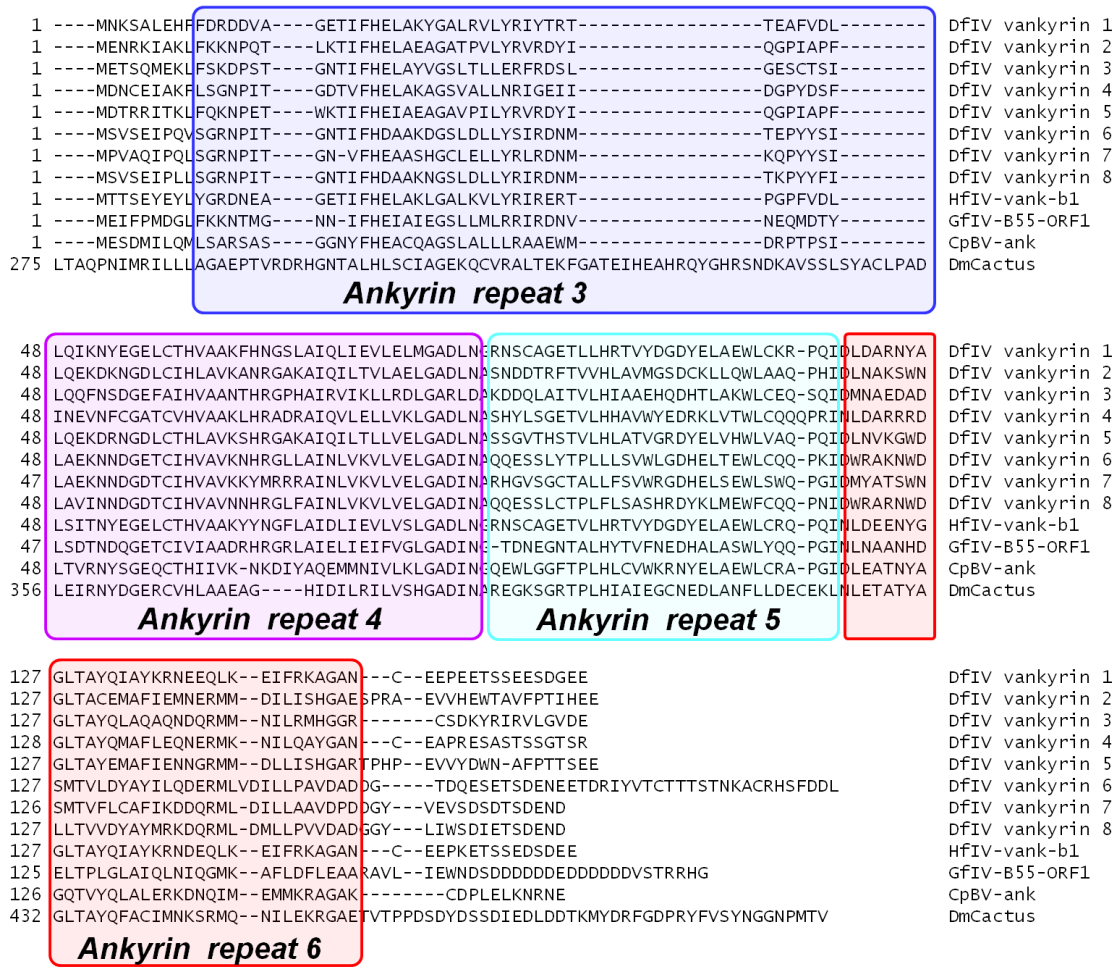


Fig. 15. Amino acid sequence alignment of DfIV *vankyrins* using the clustal W method with domain structure proofed ankyrin (DmCactus, AAA85908) from *Drosophila melanogaster* (Geisler et al., 1992) and other vankyrins from HfIV (HfIV-van-b1, AAX24120), GfIV (GfIV-B55-ORF1, YP001029391) and CpBV (CpBV-ank, AAZ04266). The conserved ankyrin domains were marked in blue, purple, green and red boxes. The ankyrin repeats numbered based on DmCactus with references (Lapointe et al., 2007; Michaely and Bennett, 1992; Michaely et al., 2002).

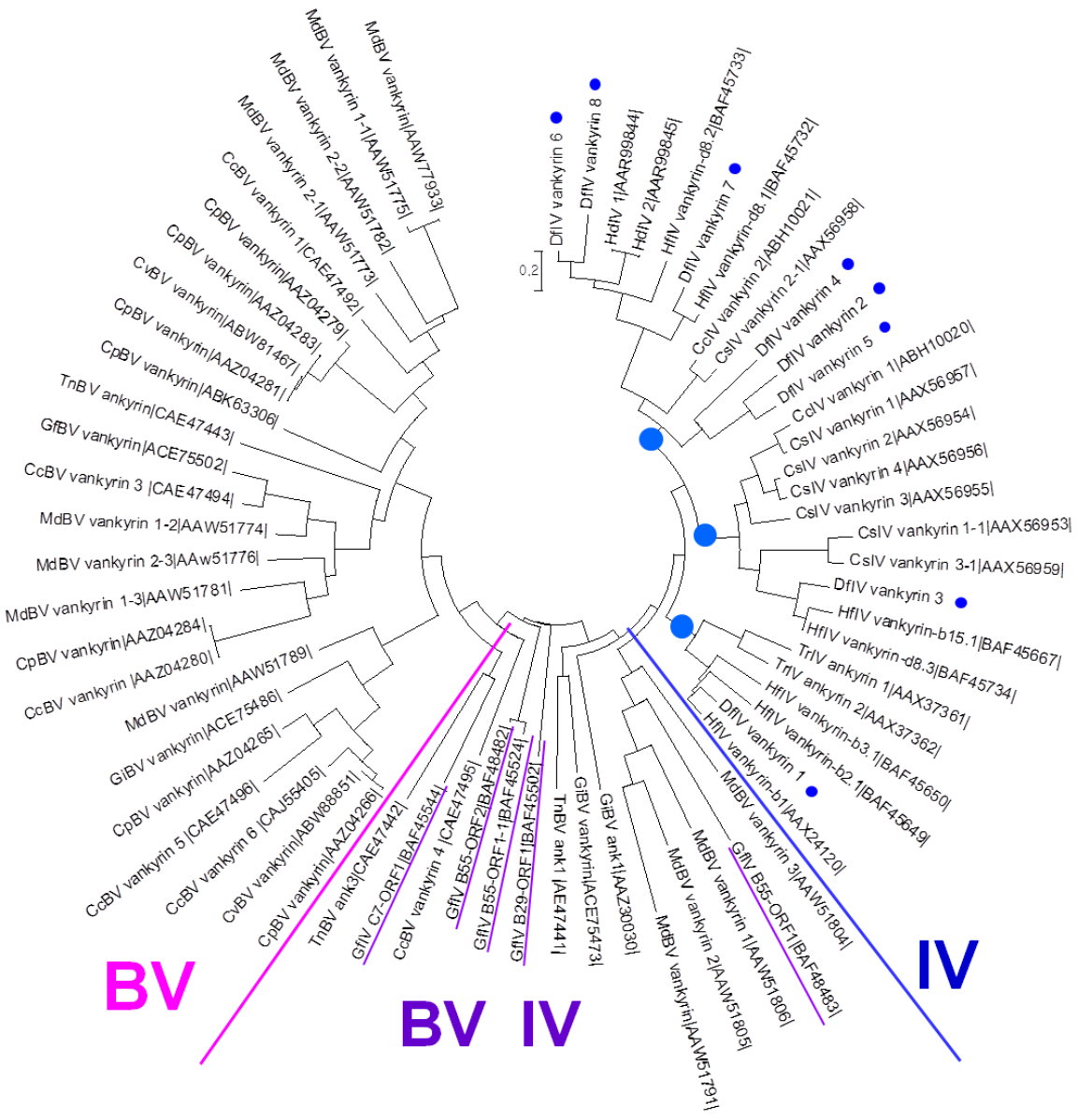


Fig. 16. Maximum likelihood phylogeny based on deduced amino acid sequences of DfIV *vankyrins* (represented by blue dots), and that of other PDVs. Blue bars signify IV orthologous while pink bars indicate BV orthologous, IV paralogous *vankyrins*. Purple bars denote IV and BV co-homologous vankyrin genes. Large blue dots represent main divergent points in IVs. The tree was produced using MEGA 5.2 with the cluster W method. The scale indicates percentage of divergence.

Fig. 17. Amino acid sequence alignment of DfIV *vinnexins* using the clustal W method with domain structure proofed innexin from the pfam database (pfam00876, NCBI) (Phelan et al., 1998). *Vinnexin* domains were marked using purple boxes. Innexin domains were predicted by DELTA BLAST and the CDD program (http://www.ncbi.nlm.nih.gov/Structure/cdd).

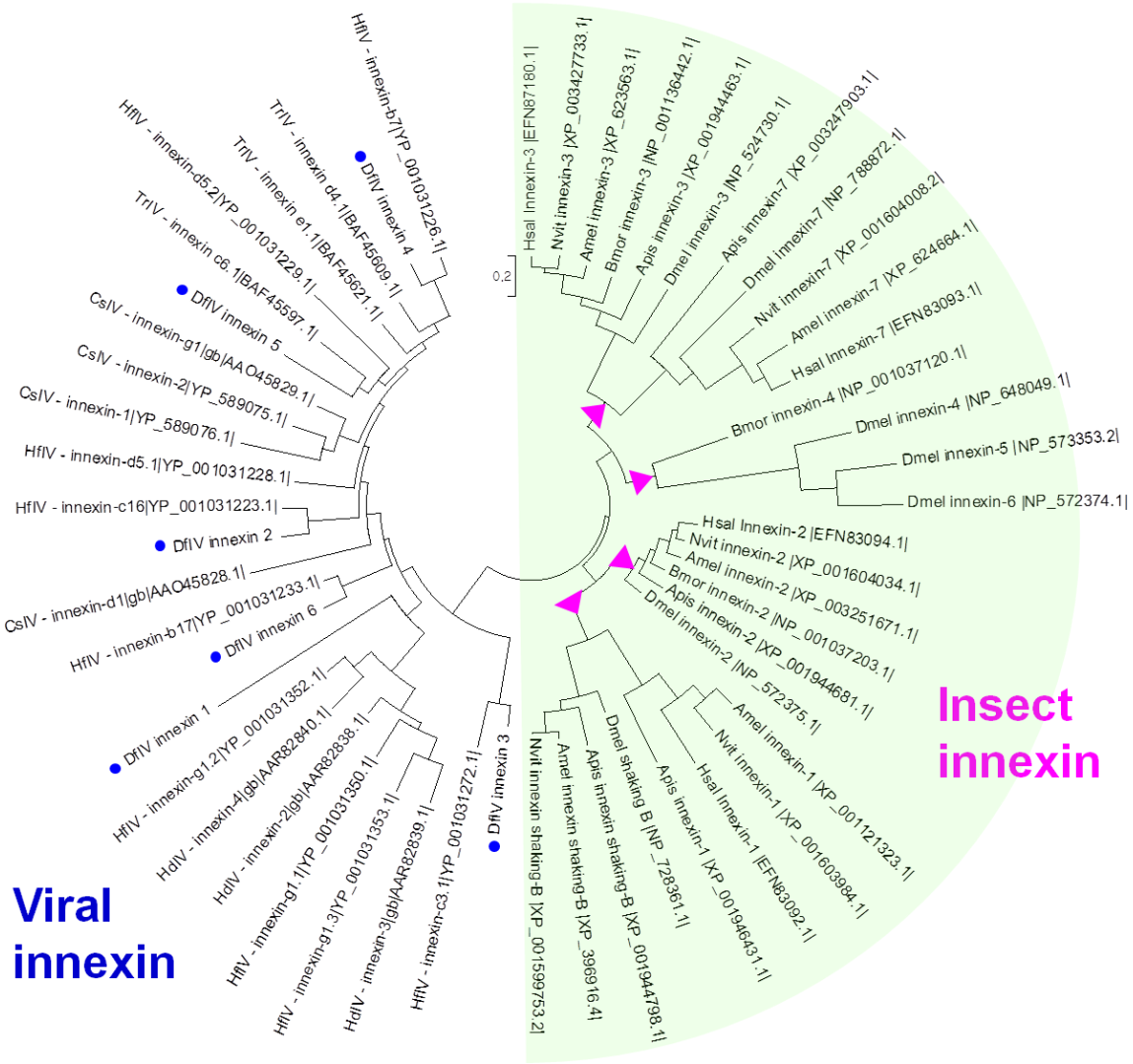


Fig. 18. Maximum likelihood phylogeny based on deduced amino acid sequences of DfIV *vinnexins* (indicated by blue dots) along with other innexins from other IVs and that of insects (Amel, *Apis mellifera*; Apis, *Acyrthosiphon pisum*; Bmor, *Bombyx mori*; Dmel, *Drosophila melanogaster*; Hsal, *Harpegnathos saltato* and Nvit, *Nasonia vitripennis*). The green half-circle indicate IV paralogous insect innexins. Pink triangle means branch point in insect innexin. The tree was produced using MEGA 5.2 with the cluster W method. The scale indicates percentage of divergence.

4. Discussion

Twenty-one ichnovirus IVs are registered in ICTV in 2012. However, more IVs have been reported in papers such as *Tranosema rostrale* ichnovirus (TrIV) (Rasoolizadeh et al., 2009), *Hyposoter didymator* ichnovirus (HdIV) (Clavijo et al., 2011), *Campoletis chlorideae* ichnovirus (CcIV) (Tian et al., 2007) and *Apophua simplicipes* ichnovirus (AsIV). Totally more than 30 IVs are reported so far. Here I report a novel IV that was isolated from *D. fenestrale*, which was first identified in *Diadegma* genus in Korea (Choi et al., 2013). DfIV showed typical IV characteristics in morphology of double membrane structure, segmented genome and genes. 99 ORFs identified from 247,191 bp of DfIV genome and these genes showed high similarity to other IVs which isolated from Campopleginae, particulally HfIV. However, another IV group which isolated from Banchine, GfIV and AsIV gene family compositions was very different and GfIV sequence similarty was also low. Untill now, AsIV sequence information can not be obtained from genbank. Some paper reported that GfIV categorized new polydnavirus, not IV (Lapointe et al., 2007). However *G. fumiferanae* and *A. simplicipes* are member of Ichneumonidae and they have some typical IV features (Djoumad et al., 2013b). It's a kind of indirect evidence of coevolution between parasitoid and PDV. This is because IVs' genome similarities were close related in parasitoid phylogeny (Espagne et al., 2004; Wagener et al., 2006).

DfIV genome informations would bring insights into ultimate understanding about coevolution between parasitoid and PDV particulary, initial question in my study. Why *D. fenestrale* suervial rates were different in two lepidoptarn hosts? However, the PDV genomes are very complicated to be fully sequenced because the genome is segmented, sometimes nested and internal sequence homologies appear between segments (Federici and Bigot, 2003; Webb, 1998.). First, the number of genome segments estimatimation was very confused. DfIV genome draft was started 120contigs based on pyrosequencing results. Accessional PCR for gap filling and sequence conformation, 67

segments were obtained. But two segments (3,000 and 2,355bp) were showed doubtful BLAST result. Any ORF sequences do not match in Genbank DB. Therefore, I conclude that DfIV has at least 65 genomic segments. Second, partial sequence similarity in segments makes trouble to align the sequence for assembly. Third, abundance segment or mixtures of segments were also interrupting (Beck et al., 2007). As with other PDVs, DfIV genomic segments vary in abundance (Chen et al., 2011) and nested segments also found (data not shown). Nowadays NGS-based genome sequencing strategy adopted (Burke and Strand, 2012; Liu et al., 2011; Wang et al., 2009). Sometimes new techniqs also applied such as plasmid capture system (Choi et al., 2009c; Choi et al., 2005). However, each method have their limits, therefore I combined NGS and PCR with Sanger sequencing.

After full or partial genome sequencing, PDV researches focused on not only each gene's functional identification (Bae and Kim, 2009; Barandoc et al., 2010; Clavijo et al., 2011; Cui and Webb, 1996; Djoumad et al., 2013a; Gad and Kim, 2008, 2009; Kroemer and Webb, 2005) but also (Bezier et al., 2009a; Bigot et al., 2008; Djoumad et al., 2013b; Dupuy et al., 2006; Espagne et al., 2004; Federici and Bigot, 2003). Moreover, some researchers tried to apply the identified gene for other fields (Gill et al., 2006) or symbiotic aspects between PDVs and parsitoids (Strand and Burke, 2012, 2013). Add to that polymerphism and comparative PDV genomics also reported (Stoltz and Xu, 1990; Tanaka et al., 2007).

DfIV genome was identified for the first time and determined as true IV. The 99 genes functions are unclear. Therefore, further study will focused on their functions and expression patterns. *D. fenestrale* is a well known generalist. Using those characteristics, evolutionary aspect also could be identified those relationships between lepidopteran hosts and DfIV with *D. fenestrale.* This is the beginning point of DfIV investigation.

Chapter Ⅱ.

Comparison of DfIV Gene Expression Patterns in Two Lepidopteran Hosts

Abstract

The genus *Diadegma* is a well known parasitoid group and some are known to have symbiotic virus, PDV. A novel IV was discovered from the calyx of *D. fenestrale* female. *D. fenestrale* has more than two hosts, including PTM and DBM. The oviposition and survival rate results showed that *D. fenestrale* preferred PTM to DBM as hosts. Nevertheless, the developmental period and morphology of *D. fenestrale* were not significantly different between PTM and DBM. To identify these phenomena, DfIV genome expression patterens were compared between PTM and DBM under various conditions. DfIV genes were more widely expressed in PTM than in DBM after parasitized by *D. fenestrale*, particularly at the initial point. In addition, large numbers of DfIV genes were expressed only in PTM and they showed differential expression patterns between two lepidopteran hosts. This DfIV genome expression plasticity showed a dependency on the lepidopteran host species and parasitization time, suggesting that it may contribute to the parasitoid survival rate increase. This may be one of the key elements that determine the symbiotic relationship between PDV and parasitoid.

Key words: *D. fenestrale, D. fenestrale* ichnovirus (DfIV), genome, *P. operculella, P. xylostella*, host preference, expression plasticity

1. Introduction

Parasitoids occur in seven holometabolous orders of insects, including Hymenoptera (Godfray, 1994; Pennacchio and Strand, 2006). Successful parasitism by insect parasitoids is a complex procedure. The parasitoid must choose the host, evade or overcome the host immune response, and adapt to or regulate host physiology to satisfy the metabolic, nutritional, and ecological needs of the larval parasitoid (Brodeur and Boivin, 2004). Among six orders, Hymenoptera has the largest number of parasitoid (Brodeur and Boivin, 2004; Pennacchio and Strand, 2006). Certain parasitoids from the Braconidae and Ichneumonidae families have developed an extraordinary strategy to protect their egg and larva from the host's immune responses (Strand and Pech, 1995). These parasitoids employ several factors that can regulate female reproductive system, including the venom, ovarian proteins, and symbiotic virus, PDV. About four decades ago, PDVs were first discovered from some parasitoid calyx fluid using electron microscope and classified as a polydnaviridae (Krell and Stoltz, 1980; Krell et al., 1982; Stoltz et al., 1988; Stoltz et al., 1976). Previous studies have shown that, in many cases, PDVs alone or in conjunction with other factors actively suppress host immunity (Edson et al., 1981; Luckhart and Webb, 1996). That means that PDVs contribute to the survival of parasitoid in its hosts, such as lepidopteran caterpillar (Stoltz and Vinson, 1979).

D. fenestrale is known as a generalist (Hardy, 1938) and it has more than two lepidopteran hosts such as PTM and DBM in Korea (Kim et al., 2012). *D. fenestrale* was initially collected from parasitized PTM infesting potato cultivation field in Jeju, Korea in May 2009. Moreover, *D. fenestrale* was also collected from parasitized DBM infesting cabbage nearby potao cultivation field. Nevertheless, the emergency rate of *D. fenestrale* from field collected PTM larvae was more than two-fold higher than that of DBM (Kim et al., 2012). Therefore, this finding led to ask following questions: why does *D. fenestrale* prefer PTM to DBM and why is the parasitism success rate

higher in PTM?To understand this host preference or parasitism success rate of *D. fenestrale*, I focused on the characterization of PDV and its gene expression patterns.

In this study, to investigate the successful parasitism rate difference of *D. fenestrale* in two lepidopteran host, progressive transcriptional profiles of DfIV and its hosts following parasitization, deep sequencing-based transcriptome analyses and qrt-PCRs were carried out for parasitized or non-parasitized larval samples of PTM and DBM. This study would contribute to the understanding of host-specific gene expression patterns of PDV.

2. Materials and Methods

2.1. Insects

2.1.1. Parasitoid

D. fenestrale was initially collected from parasitized PTM infesting potato cultivation field in Jeju, Korea in May 2009 and has been maintained in the HARC insect rearing room. *D. fenestrale* was reared on PTM and DBM as hosts in plastic cages (30 cm, cube shape) under the conditions of 25±2℃, 16 L : 8 D photoperiod, and 50-70% relative humidity. Third instar PTM or DBM larvae (5 and 3 days after hatch, respectively) were parasitized by *D. fenestrale* in an open-type cylindrical plastic cage (15 cm diameter, 30 cm height) for 24 h and parasitized hosts were reared in the same condition as the unparasitized larvae until emergence. The emerged *D. fenestrale* adults were collected everyday and allowed to mate for 24 h before use for parasitization. Adult wasps were fed with 10% sucrose solution.

Cotesia glomerata (Hymenoptera: Braconidae: Microgastrinae) was collected from parasitized DBM larvae in Daegwallyeong in July. 2007. *C. glomerata* was reared on DBM using the same method as that of *D. fenestrale*.

2.1.2. Lepidopteran hosts

The PTM larvae were collected from Jeju, Korea, together with parasitic wasp, *D. fenestrale*. The emerged PTM adults were allowed to mate in an open-type cylindrical plastic cage (15 cm diameter, 30 cm height) with a filter paper on the top for oviposition. The PTM eggs attached to the filter paper was transferred plastic cage (30 cm, cube shape) with potato tuber or plant (*Solanum tuberosum*). PTM was reared in the same cage until adult stage or third instar larva to use as a wasp host. The DBM larvae were collected from Daegwallyeong in 2007. Larval stage of DBM was

reared in Napa cabbage (*Brassica pekinensis*). DBM pupae were collected and held in an open-type cylindrical plastic cage with crumpled aluminum foil treated with cabbage extract solution for oviposition. Cabbage extract solution was made from autoclaved cabbage with water (1:4 ratio, weight / volume) and filtered by filter paper. DBM and PTM eggs were used immediately or stored at 4℃ for a month until use.

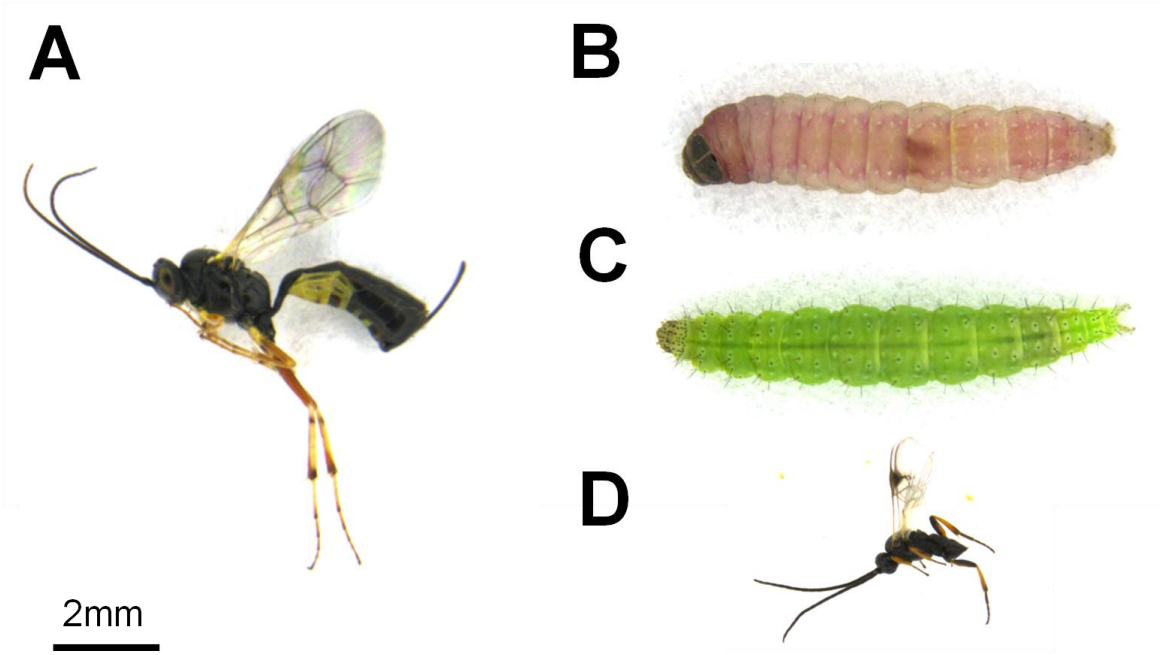


Fig. 19. The parasitoid wasp, *D. fenestrale* (A, female adult) and its two lepidopteran hosts (B, *P. operculella*, and C, *P. xylostella*) were used in this study. Another parasitoid wasp, *C. glomerata* (D, female adult) was used as a reference parasitoid in comparing developmental period of lepidopteran hosts with or without parasitization.

2.2. Developmental characteristics of *D. fenestrale* in two lepidopteran hosts

2.2.1. Comparison of *D. fenestrale* developmental period in two lepidopteran hosts

In the preliminary experiment, *D. fenestrale* was reared in the same cage with some other lepidopteran larvae at young stages. *Spodoptera exigua* and *Mamestra brassicae* were not parasitized but DBM was parasitized by *D. fenestrale*.

Comparison of *D. fenestrale* developmental period between PTM and DBM was performed as wasp rearing methods as describe above. In brief, 3rd instar larvae were parasitized or left unparasitized for 24 h by placing the larvae into the cages with or without *D. fenestrale*, respectively, and then > 30 larvae were collected and reared in a single individual dish (5.5 cm diameter) to check the individual developmental period until emergence. *D. fenestrale* was confirmed to parasitize both PTM and DBM, whereas *C. glomerata* parasitized only DBM as expected. All experiments were replicated four times for both PTM and DBM. Statistical analysis was conducted by SAS 9.1 (SAS institute Inc., Cary, NC, USA).

The developmental characteristics and developmental periods of *D. fenestrale* were observed after dissection of host larva under a Leica M205C stereomicroscope and photographed with a DFC450 camera system (Leica, Wetzlar, Germany) on a daily basis. A total of more than 1,000 larvae were dissected in five replications for each host.

2.2.2. Morphological characteristics of *D. fenestrale*

The morphological characteristics of *D. fenestrale* were observed after dissection of host larva in larval stage under a Leica M205C stereomicroscope and photographed with a DFC450 camera system (Leica) on a daily basis. After pre-pupal stage, *D. fenestrale* were observed directly or after remove the pupal silk.

2.2.3. Host preference of *D. fenestrale*

Host preference of *D. fenestrale* between PTM and DBM was performed as wasp rearing methods as describe above. In brief, 100 of 3rd instar PTM and DBM larvae were parasitized for 24 h by placing the larvae into the cages with 30 pairs of *D. fenestrale*, respectively, and then 30 larvae were collected in random and dissected under a Leica M205C stereomicroscope (Leica) for parasitic rate analyzed. 30 larvae were also randomly picked up and reared in each cages to check the survival rate until *D. fenestrale* emergence. All experiments were replicated three times.

2.3. Transcriptional profile comparison of DfIV genes between two lepidopteran hosts

2.3.1. Deep sequencing-based transcriptome analysis of DfIV genes and hosts genes

After 24-h parasitization period, > 70 larvae of parasitized or unparasitized lepidopteran hosts were randomly chose and dissected to collect lepidopteran host tissues only on slide glass. The parasitized host samples were designated as PTM–Df 1 or DBM–Df 1 (one day after parasitization by *D. fenestrale*) whereas corresponding unparasitized host samples as PTM 1 or DBM 1 (same aged unparasitized larvae from the same parents as PTM–Df 1 or DBM-Df 1). Likewise, host tissue samples of PTM–Df 3, 5 or DBM–Df 3, 5 (3 or 5 days after parasitization by *D. fenestrale*) and PTM 3, 5 or DBM 3, 5 (corresponding unparasitized) were also prepared. The PTM 1, 3, 5 or DBM 1, 3, 5 samples were mixed to prepare respective pooled unparasitized host control. Finally, respective host samples with DfIV injected without parasitization were prepared, in which unparasitization was confirmed by both microscopic examinations of dissected larvae and PCR with DfIV marker gene primers. As a result, a total 10 different samples were prepared for transcriptome analysis: PTM or DBM–Df 1, 3, 5 (1, 3, 5 days after parasite by *D. fenestrale*, respectively), PTM or DBM–DfIV (un-parasitized but DfIV injected by *D. fenestrale*) and PTM or DBM (unparasitized, same stages of PTM or DBM-Df 1, 3, 5 mixed, mainly 4^{th} instar larvae). Total RNA was extracted from these samples with suitable volume of TRI reagent (MRC) according to the manufacture's protocols. Total RNA samples were sent to Macrogen to run whole transcriptome shotgun sequencing (RNA seq). RNA samples quality was confirmed by 2100 bioanalyzer RNA 6000 NANO chip (Agilent Technologies, Waldbronn, Germany). Using TruSeq sample preparation kit (illumina, San Diego, CA, USA), sequencing library was constructed from total RNA through sequential procedures, including purification and fragmentation of mRNA, first strand cDNA synthesis, second strand cDNA synthesis, end repair, addition of "A" bases to 3' ends, ligation of adapters, purification of ligated products, and PCR amplification to enrich cDNA templates. The

library was validated, quantified and subjected to deep sequencing using Hiseq 2000 system (illumina). The *de novo* transcriptome assembly was performed by Trinity (Grabherr et al., 2011). Digital expression profile analysis was also conducted by Trinity. Functional annotation of putative protein databases from the assembled contigs was conducted according to the Gene Ontology (GO) database (http://www.geneontology.org). The BLAST-NR search was then performed against NCBI NR. Quantifying expressions were normalized using RPKM (reads per kilo base per million) (Mortazavi et al., 2008).

2.4.2. Quantitative real time PCR (qrtPCR)-based gene expression analysis of DfIV genes

The two lepidopteran host samples of various larval stages were prepared for gene expression analysis and validation of transcriptome analysis using the same methods of transcriptome analysis based on RNA-seq. Lepidopteran larval stage was divided six steps based on the parasitoid development. Sample name larva 1 to 6 means that *D. fenestrale* stages were: 1 – egg, 2 – 1^{st} instar, 3 – 2^{nd} instar, 4 – 3^{rd} instar, 5 – early 4^{th} instar and 6 – 4^{th} instar. All larval steps were sampled from three treatment groups: unparasitized, parasitized and un-parasitized but DfIV injected by *D. fenestrale* were also prepared. 18 total RNA samples were prepared from each host in larval stage (6 steps based on *D. fenestrale* developmental stages with 3 treatments). Additionally a pupa and adult samples were prepared from two treatments; unparasitized and un-parasitized but DfIV injected by *D. fenestrale*. Therefore, totally 22 RNA samples were prepared from each host as well as the DfIV genomic DNA sample was also prepared as a positive control. Totally, 45 samples were used for qrtPCR. Total RNA and DNA were extracted as describe above. Gene expression analysis was performed by DELTAgene™ assays system (Fluidigm, South San Francisco, CA, USA) with qrtPCR primer sets (Table 3). Quantitative analysis was conducted by relative quantification method modified from the original concept of $2^{-\Delta\Delta Ct}$ methods (Pfaffl, 2001). DfIV genome segments' copy numbers were relatively calculated by *cys-motif* 2 gene expression level.

Table 3. Primers used for qrtPCR

Target	Product length	Forward	Reverse
DBM 18S rRNA	146	ACGAACATCAGCGAAAGCA	GAGCCATTGTAGTAACGTC
Df 18S rRNA	113	GACTCAACACGGGAAACC	TCGCTCCACCAACTAAGA
PTM 18S rRNA	146	ACGAACATCAGCGAAAGCA	GAGCCATTGTAGTAACGTC
DfIV- rep 01	112	ACTGGTTGAACTTCTACTTGAC	AGGTGGACCGTGTTACTT
DfIV- rep 02	96	AACGATGAGGACCAGATGA	AAGGTGATCCGAACAGTAATG
DfIV- rep 03	110	GCCAGCGGTGAATATGTT	GGTGACATGAACAGGAAGAG
DfIV- rep 04	101	AACTGTGGAGGATGTCTATC	ATCTTCTTCTATTCTTGCTGGAT
DfIV- rep 05	144	CTCGAAGCTGTCAGTGTA	GTCTGGCTCCAATGTTGA
DfIV- rep 06	134	CATCAGCAGAATCAGAATCAAC	GCTCAAGTCGTTATTCGGATA
DfIV- rep 07	96	GATGACAAGATAGCCGAGG	ACTCTCCAGCAGGTATTCC
DfIV- rep 08	179	ATACGCCTCCTGTCCCT	TGAATGACCCTTCTTCCAAAT
DfIV- rep 09	179	ATACGCCTCCTGTCCCT	CGAATGACCCTTCTTCCAAAC
DfIV- rep 10	179	ATACGCCTCCTGTCCCT	TGAATGAGCCATTTTCCAAAG
DfIV- rep 11	135	ATCTAAATCTGTGTGACAATGGT	GCGAGCAATAATGGTGGAA
DfIV- rep 12	102	TGTGGAAACTTTACTACCGATAAC	AGACGATGTCACTCAGTTT
DfIV- rep 13	131	AACAATGCCGCATCAACTA	CACCCTCACGAAATCTCTTT
DfIV- rep 14	128	AAGTATGCACTGTCCGTTA	CACCCTCACGAAATCTCTTT
DfIV- rep 15	184	GCCACACGAACAGAAGAA	TGCCACAGTAACAGGAGT
DfIV- rep 16	91	CTCCGTGGTCAAACTATTCAA	AACAATGCTGGTCTTCTTCTT
DfIV- rep 17	128	AGCATCGTCTATTCGGAGTA	CAGGTGATAGGCAATGTCTT
DfIV- rep 18	117	CAAATCGTTTCCAGACAGAGA	GATATTCGTTCAGCCACAGAT
DfIV- rep 19	100	GCATTTGTAACCTGATTGGAAA	CAGCACGAGTGATGGAAG
DfIV- rep 20	137	GACAGAACTACACAACTTGGTA	CGGCAGTGAAGTGATACG
DfIV- rep 21	140	TGTGGCAACTATCAACTCATC	CGAACCGAACACTGGAAG
DfIV- rep 22	152	CGAACAAATCGCCGAGC	AGTCAACAATCATCACATCATAGT
DfIV- rep 23	88	CGGTGACTACGCTTCTTG	ATGCCAGTGATTATCTTGACAA
DfIV- rep 24	125	GGATGTATGTTCGGACTATCG	AGTGATGGAAATGGTTGTGTT
DfIV- rep 25	170	GCATCAGTTATCCGCTTCC	GAGTAGACATTCGCCATAGTG
DfIV- rep 26	91	GCCATCACCTGCTACTTC	CCAACGCTTAGACTTCCAA
DfIV- rep 27	131	AGCATACCTCCTACCATTGT	CATACGCCACTGGATAAGAG
DfIV- rep 28	102	GTGAACGAACATTTGGAGC	GGAGTTGCCGAATAGTCCTT

Table 3. (Continued)

Target	Product length	Forward	Reverse
DfIV- cys motif 1	109	CAACAACCGCACTGATCTATA	AACGAGGAGAAATACCAAAGAG
DfIV -cys motif 2	82	AGCAGAAGCAGAGTGTCA	CTTGGTAGAATGTGAGCAGTT
DfIV- cys motif 3	229	AATTACGCTGATTGCTTGGA	CGAGTGTCTGATGATACTGTTT
DfIV- cys motif 4	139	AAGTATGCTCAGTTAGTTAAGGTT	CATCATCACGCTCTATTGGAA
DfIV- cys motif 5	238	TTGTGCCAACCATTATATGAGAT	CTGTATCGTTAGGAATCATCTGT
DfIV- cys motif 6	101	TCTGATAACACCGTAACAACAA	ATCGCATTGGAATCTGTGAA
DfIV- cys motif 7	250	AGCAACCAACTTCACAGATT	CATAGGAGTGTCAGGAATCG
DfIV- cys motif 8	209	TACTTCGTTCGTTGCTGTC	ATACACTCCCACCTTTCTGA
DfIV- vankyrin 1	139	TTCACAACGGGTCTCTTGCA	GCCAGCTCGTAATCTCCA
DfIV- vankyrin 2	144	TGGTACATCTGGCAGTCAT	CCATCATCCGTTCGTTCAT
DfIV- vankyrin 3	150	GCTATTACCGTACTACACATCG	CATCCGTTGGTCGTTCTG
DfIV- vankyrin 4	77	CGTGCCATACAAGTGTTAGA	GTTTCGCCTGAGAGATAGTG
DfIV- vankyrin 5	110	AACTGGTTCATTGGTTGGTC	CATCATACGTCCGTTGTTCT
DfIV- vankyrin 6	157	TCCTCACTCTATACTCCACTTC	GCATCCGTTCGTCTTGTAA
DfIV- vankyrin 7	115	ACGGTATTTCTGTGTGCTTT	CATCACTTGTATCACTGTCACT
DfIV- vinnexin 1	143	GCAGCGAGAAGAACAGAA	GAACATATCCATCAGCACCAT
DfIV- vinnexin 2	125	AAGTGACAGTGATCCTTCTTG	GATGTAGCAGTAGGTGTTGAG
DfIV- vinnexin 3	196	CATCTGGCAATCACTGGAA	GGTCATAACATTCGTCAAGTTG
DfIV- vinnexin 4	173	TTCCTTGGCAACGAGTTC	AGTCCGTCTCTTAGTTCTACA
DfIV- vinnexin 5	126	AGAATGAAGATGTTGGCTGAG	GTAGGCATAACTGTTGTGAGAA
DfIV- vinnexin 6	75	ACTGAAGATATTGACTCACGAG	ATATGGTTGACCTTCTCTGTG
DfIV - polar residue rich 1	119	ATATTACCTGCGGCAAGATG	CTCTACGGCTCTCCTCAG
DfIV - polar residue rich 2	192	AGGAACAAGAAGCCAGGA	ACACCTCCGCCATTATCT
DfIV- thr-ser like 1-1	199	TTCTGCTGATCTTGGTGGT	CTGCCTGTAGTGGATCTG
DfIV- thr-ser like 1	100	CCGACTTCTACAACTGAAG	GCCTTCTGGGTGGTAAGG
N gene like	135	ACGGACAACATAGCAATCG	AGAAGCGGTGAGTTCAGA
GfIV c7 like	161	TTGGTCCTTGGATGTAGTCA	CGCTCTGAATCGGTTGTG
HdIV p12 like	88	TGATGACTTTGGTTCTGATGG	CGGGTAGGATCGGTGAAA
DfIV c57 like	155	AGTTCCTTCGTCGGTTGA	CAGCAGGTACACATGATGAT
HfIV c10.1 like	99	TTGATGAAGGTTACAGCAGTT	CGCAGAAGTATGAGAGCC
HfIV c12.1 like	146	TTCAAGAAGCGGCGTTAC	TCAGACTCATCGGAAGACAT

Table 3. (Continued)

Target	Product length	Forward	Reverse
HfIV c17.1-1 like	246	TAGCAGCCGAAGACCATT	ACACAGTAGCCACCAGAT
DfIV c20	186	CGGCAATCATCACAACCT	GGAACAGAATCTTATCCTCACAG
N-kinase like	94	CTATCCTATCGCAAAGCACAA	TCCCGCAATCCTAATCCA
HfIV c17.2-1	91	CCGAGCGTGTAGATGATTC	GACCGTTGGTTGGGATATG
HfIV c17.2 like	161	CTCCGAAGGTATGAATGAAGG	ACTCTCCATAACTCCACGAA
HfIV b7.1 like	162	GAAGAATGTCGTCGTAATGAGA	TCGCTTGATGGAGGATGA
GET like	170	CCTCGTATGCCGTGTAATC	ATCTTTGCTCTCCTCTCTACT
HfIV b7.1 like	162	GAAGAATGTCGTCGTAATGAGA	TCGCTTGATGGAGGATGA
HfIV e1.3 like	97	CAGGGCACACAGTAATGG	AGGAGGGCTTTCTTCAGT
HfIV c20.1 like	250	CTCAGATGTCGCCAGAAC	TAGCCATAGCCGCAAGAT
DNA pol 3 like	102	CCACTCAATCTATCACGGAAG	CTGGCTCGGAAGATGTTG
DNA pol 3 like2	134	TTGACGAGAATTACGAAGAACA	CAGCAGCAGTCTTGATGT
HdIV 3 like	116	GTGTGGGCTTCTTTGTCA	CATTTGTCTTCTTTATCCGTATCC
SerB like	162	CTGGCTTGGAAGTAAAGGTT	AGTACGGACGCATCATCA

3. Results

3.1. Developmental characteristics of *D. fenestrale* in two lepidopteran hosts

3.1.1. Comparison of *D. fenestrale* developmental period between two lepidopteran hosts

The rate of parasitism was 10 to 30 % against PTM in the field condition when surveyed in the potato fields, Jeju, Korea, from 2010 to 2012 but that of DBM was lower than 10 %. The parasitism rates were dramatically increased in a laboratory condition up to 70 % in both cases of PTM and DBM. This was likely to be due to the provided optimal oviposition time point the parasitoid to lepidopteran hosts (5 and 3 days after hatching, respectively) (Kim et al., 2012).

Developmental periods of *D. fenestrale* to different lepidopteran hosts were compared with those of another parasitoid, *C. glomerata,* as a reference, which is known as a parasitoid to DBM and has its specific PDV, CgBV (Barandoc and Kim, 2009). *D. fenestrale* was normally grown in two different hosts. The developmental period in all stages and also their life spans were not significantly different ($p > 0.05$, T-test) between two hosts (Fig. 20). However, the average life span of *D. fenestrale* grown in PTM was prolonged a day compared to that of DBM, especially in larval stage (about 0.6 day). The period from the onset of parasitism to pupation was designated as larval stage and the larval period of *D. fenestrale* was 8-9 days at 25℃ (Fig 20).

The larval developmental periods of PTM and DBM were extended to 1-2 and 2-3 days after parasitization, respectively. The larval periods of unparasitized PTM and DBM were 7 and 5 days, respectively. As a result, regardless of different hosts, *D. fenestrale* appears to regulate the developmental period of its lepidopteran host for its own survival. This phenomenon was also observed in DBM parasitized by *C. glomerata*. In this case, DBM's larval stage period was significantly prolonged after parasitization. Taken together, developmental period of each lepidopteran host was differently regulatedby parasitoid.

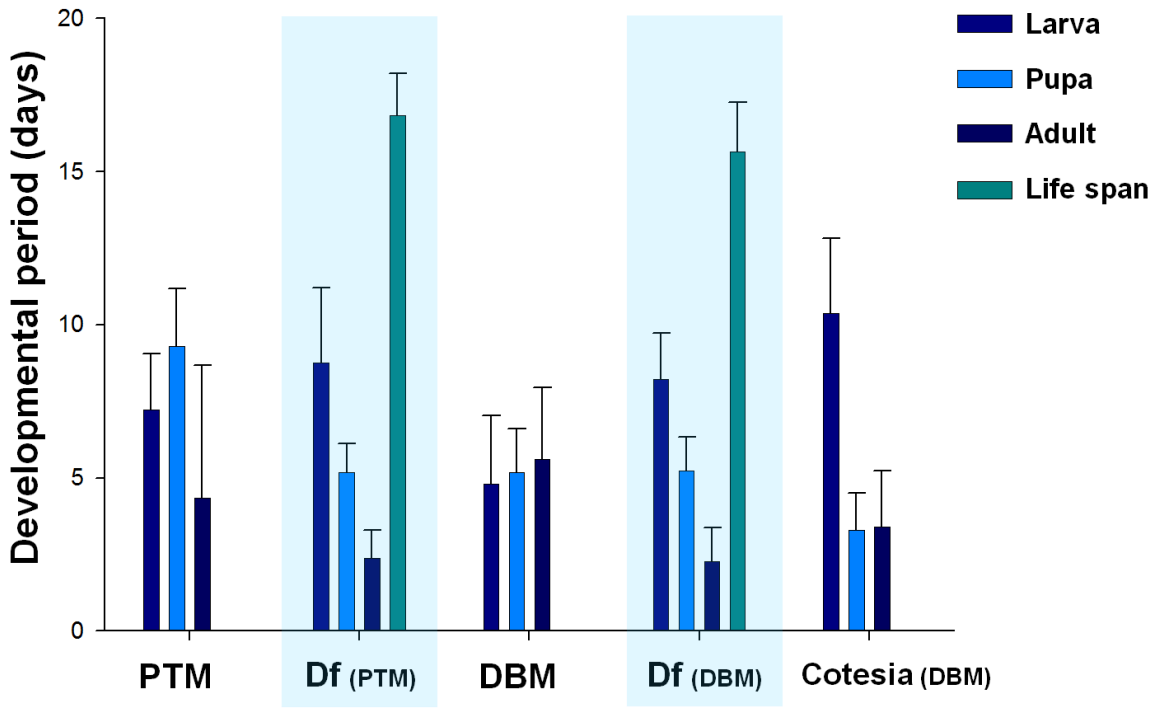


Fig. 20. Developmental periods (larva: oviposition to cocoon forming, pupa: cocoon forming to emergence, adult: emergence to die and life span: sum of all stage periods) of parasitic wasp *D. fenestrale* (Df) in two lepidopteran hosts, *P. xylostella* (DBM) and *P operculella* (PTM). All experiments were replicated four times in both PTM and DBM (n = 30 in each replicate). Error bar means standard deviation.

3.1.2 Morphological characteristics and host preference of *D. fenestrale*

D. fenestrale was dissected from two hosts and their developmental and morphological characteristics were compared for better understanding of *D. fenestrale* developmental physiology in two hosts. Nevertheless, there was no difference in morphology and developmental periods of *D. fenestrale* regardless of its host. Only *D. fenestrale* which developed in DBM larval period was about half day shorter than that of PTM, but it was not significantly different because of individual variations (Fig. 20). Newly deposited eggs were white and arcuate Fig. 21A). After maturation with segmentation (Fig. 21B), about two days after oviposition, 1st instar larva was hatched from egg (Fig. 21C). In this study, four larval instars were recognizable, which is consistent with the results of *D. semiclausum* (Huang et al., 2009a). Larva had three thoracic segments, and 10 abdominal segments. In the first three instars, they had a enlarged head with tapered body and cauda (Figs. 21D-F). The body was colorless and transparent in the 1st instar, with only some trachea visible in white (Fig. 21D). In the 2nd instar, the tracheal system was visible through the integument; the gut was visible, and its color turned from yellow (Fig. 21E). In the 3rd instar, body size increased and the gut was filled with digested host tissue. Simultaneously, the cauda shortened but still significantly remained (Fig. 21F). The 2nd and 3rd instars also could be distinguished by their head shape; only 2nd instar had a node in their head. At the 4th instar stage, the spindle-shaped body was dramatically enlarged and the cauda was almost undetectable (Fig. 21G). At the 4th instar stage, parasitoid larvae consumed all the organs and tissues of the host except the cuticle. Pupal stage was divided into three stages. Approximately 8-9 days after oviposition the late 4th instar larva began to spin (Fig. 21H). Body became crumpled and turned yellow in the 1st pupal stage (Fig. 21I). Eyes were observed and body cocoon color changed in the 2nd pupal stage (Fig. 21J), where typical shape of wasp was observed (Fig. 21K),

As these results, *D. fenestrale* parasitized two lepidopteran hosts, PTM and DBM. However, their parasitic rate and survival rate were different two hosts. *D. fenestrale* was parasitized 91.7 % in

PTM and 73.3 % in DBM, in average. *D. fenestrale* was survived 83.3 % in PTM and 46.7 % in DBM, respectively. Oviposition rates (number of eggs) were 3.2 eggs/larva in PTM and 1.1 eggs/larva in DBM. Therefore, PTM was a better host in *D. fenestrale* survival.

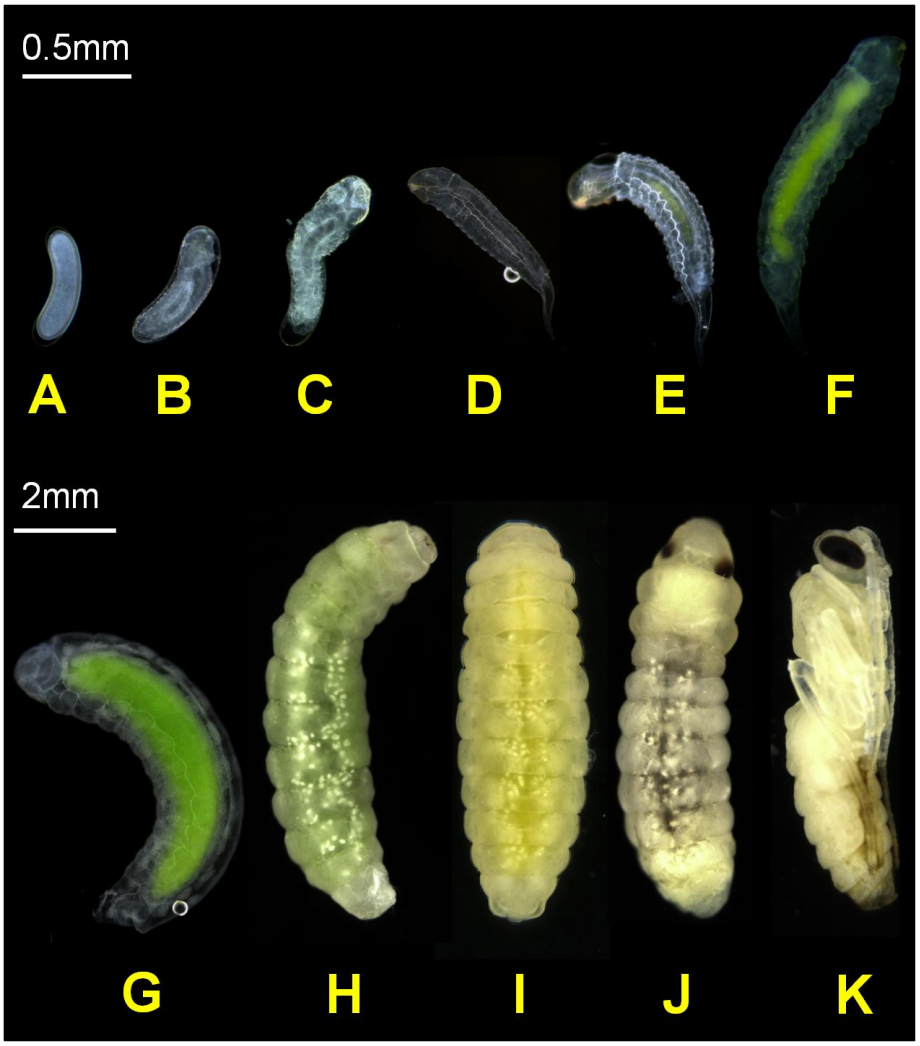


Fig. 21. Typical morphologies of *D. fenestrale* egg; 24 hours after oviposition (A, 1 day), before hatching (B, 1-2 days) hatching (C, 2 days), 1st instar larva (D, 2 days), 2nd instar larva (E, 3days), 3rd instar larva (F, 4 days), 4th instar larva (G, 5 days), late 4th instar larva (H, 7days), 1st to 3rd pupal stage (I to K, 8-9 days after oviposition, respectively) dissected from parasitized DBM at different time points or cocoon. Scale bar = 0.05mm (A – F) and 2mm (G – K).

3.3 Transcriptional profile comparison of DfIV genes from lepidopteran hosts

3.3.1 Deep sequencing-based transcriptome analysis of DfIV genes from lepidopteran hosts

In this study, Hi-seq2000-based RNA-seq was performed to identify the DfIV gene expression pattern in two lepidopteran hosts. To analyze the DfIV gene expression pattern in PTM and DBM larvae following parasitization by *D. fenestrale*, RNA samples isolated from the host larvae (1, 3, 5 days after parasitization; PTM or DBM_Df 1, 3, 5), (unparasitized; PTM or DBM) and (unparasitized but DfIV injected by *D. fenestrale*; PTM or DBM_DfIV), respectively. Total 10 samples (5 samples in PTM and DBM, respectively) were analyzed by Hi-seq2000. However, due to low sample quality, results could not be obtained from three samples (PTM, PTM_Df 1 and DBM_DfIV). After data quality filtered through Q30, deep sequencing analysis (excluding three samples) produced approximately 101 and 97 Mb sequence information in DBM and PTM, respectively (Table 6).

A total of 99 genes were predicted based on DfIV genome annotation. These gene expressions were analyzed after transcriptome results were normalized. All RNA-seq read fragments were mapped onto the DfIV genes and read counts were calculated after mapping. The gene expression level was calculated using RPKM (reads per kilo base per million) values from the read count calculation. Contig assembly and BLAST were also produced. Figure 22 is an overview of the four main gene families and other unassigned DfIV gene expression levels obtained from the two hosts. Among the 99 DfIV genes, DfIV *vankyrin* 1 was the most highly expressed, followed by DfIV GET like 1.(Fig. 22). The GET gene was known to be involved in the Golgi to ER traffic complex (Schuldiner et al., 2005). For a better understanding of each gene expression pattern, the samples were separately analyzed within each gene family.

The function of the *rep* is not known but it was only predicted to play an important role in viral cycles (Galibert et al., 2006). Among the 40 *reps*, only ten *reps* were significantly expressed

(>10,000 in RPKM value, *rep*3, 6, 8, 9, 10, 13, 14, 15, 18 and 19). Generally, these *reps* were expressed at a higher degree in PTM than in DBM. This was especially true for the DfIV *rep* 15, 8, and 6 (Fig. 23). *Rep* 15 and 8 were more expressed in PTM_Df 5 than in PTM_Df 3. *Rep* 8 and 16 were highly expressed in PTM_DfIV, which has only DfIV without parasitization. In summary, *reps* were differentially expressed in the two hosts, and particularly more expressed in PTM.

The function of the *cys-motif* is known to inhibit the host's cellular immune system in CsIV (Li and Webb, 1994). Twelve *cys-motifs* were expressed lower than *reps* (Fig. 23). Among them, only two *cys-motifs* (1 and 2) were commonly expressed in all lepidopteran host samples except the unparasitized sample. *Cys-motif* 4 was most highly expressed in DBM_Df 5. However, *cys-motif* 2 was two folds more expressed in PTM_Df 5 than in DBM whereas *cys-motif* 1 was almost equally expressed.In general, the extent of differential expression of *cys-motifs* between the two hosts was slightly less than that of *reps*.

The function of *vankyrin* is known to inhibit the lepidopteran host's transcription (Kroemer and Webb, 2005). Among the eight *vankyrins*, only *vankyrin* 1 was highly expressed in all samples except the unparasitized DBM. No apparent differential expression between two hosts was observed at 3 days post-parasitization but, at 5 days post-parasitization, *vankyrin* 1 was about 3 folds more expressed in DBM than in PTM (Fig. 24).

Vinnexin was known to create gap junctions in invertebrates (*innexin*) and IVs (Marziano et al., 2011; Phelan et al., 1998). Among the six *vinnexins*, only two *vinnexins* were expressed. Especially, *vinnexin* 1 was expressed in all samples except unparasitized but no apparent difference in the level of transcription between PTM and DBM at 3 days post-parasitization. However, *vinnexin* 1 and 3 were 3 folds more expressed in PTM than in DBM at 5 days post-parasitization (Fig. 24). As results, *vankyrins* and *vinnexins* were commonly expressed in the same level until 3 days post-parasitization but showed differential expression patterns between two hosts from 5 days post-parasitization.

Finally, among 33 unassigned genes belonging to four main gene families, only 7 genes,

including GET like and thr-ser like genes, were significantly expressed (Fig. 25). GET like gene was differently expressed in two hosts between 3 and 5 days post-parasitization. At 3 days post-parasitization, GET like gene was 2 times more expressed in PTM but it was more expressed in DBM after 5 days post-parasitization. On the other hands, four genes (thr-ser like 1-1, N, HdIVp12 like and HfIV c12.1 like gene) were more expressed in DBM at 3 days post-parasitization but they were more expressed in PTM at 5 days post-parasitization. However, there was no apparent tendency like the four main gene families. Furthermore, alternative splicing detected in thr-ser like genes because, two genes encoded single segments and overlapped their ORFs, only the thr-ser like 1-1 gene was expressed (Fig. 25) and HdIV p12 like gene also found their splicing (supplementary fig. 1). Alternative splicing was also reported from MdBV (Burke and Strand, 2012) and protein diversity could enhance their parasitism (Zheng, 2010).

Table 4. Results of lepidopteran hosts (DBM and PTM) RNA-seq data processing

Raw sequences				
	Read type : Paired-end			
	Read length (bp) : 101			
			No. of total reads	Total length (bp)
	DBM	DBM	179,578,058	18,137,383,858
		DBM_Df 1	77,132,380	7,790,370,380
		DBM_Df 3	194,219,136	19,616,132,736
		DBM_Df 5	94,198,968	9,514,095,768
	PTM	PTM_Df 3	82,564,302	8,338,994,502
		PTM_Df 5	93,828,522	9,476,680,722
		PTM_DfIV	86,059,316	8,691,990,916
Transcriptome Assembly				
	Total no. of contigs	Total length of contigs	Max length	Min length
DBM_merge	196,081	101,315,631	15,853	201
PTM_merge	135,771	96,888,895	27,521	201

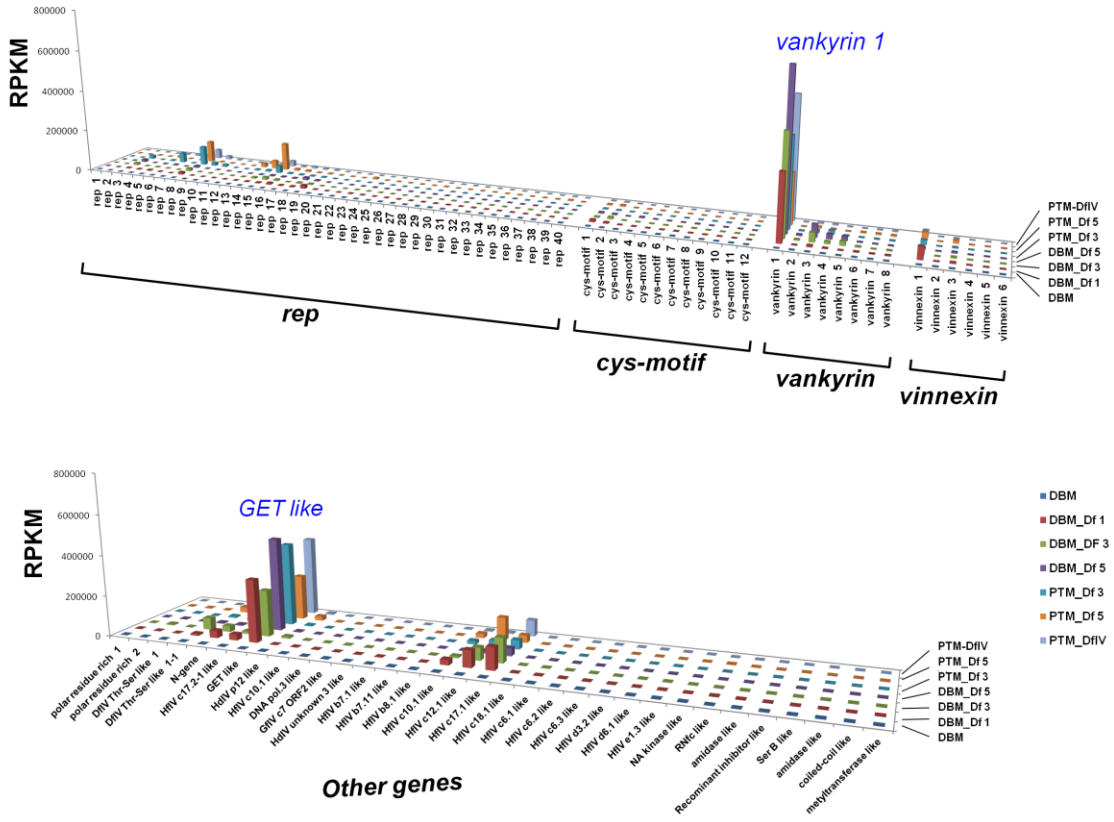


Fig. 22. Overview of four main gene families and other unassigned DfIV genes expression patternsin two lepidopteran hosts as determined by RNA-seq. Among these genes, DfIV *vankyrin* 1 was most highly expressed in both DBM and PTM. The DfIV GET like gene was also highly expressed. *Rep*, *cys-motif*, *vankyrin* and *vinnexin* families were analyzed below. RNA samples isolated from the host larvae (1, 3, 5 days after parasitization; PTM or DBM_Df 1, 3, 5), (unparasitized; PTM or DBM) and (unparasitized but DfIV injected by *D. fenestrale*; PTM or DBM_DfIV), respectively. Total 10 samples (5 samples in PTM and DBM, respectively) were run Hi-seq200. However, due to low sample quality, results could not be obtained from three samples (PTM, PTM_Df 1 and DBM_DfIV). All RNA-seq read fragments were mapped in DfIV genes and read counts were calculated after mapping. The gene expression level was calculated using RPKM (reads per kilo base per million) values from the read count calculation.

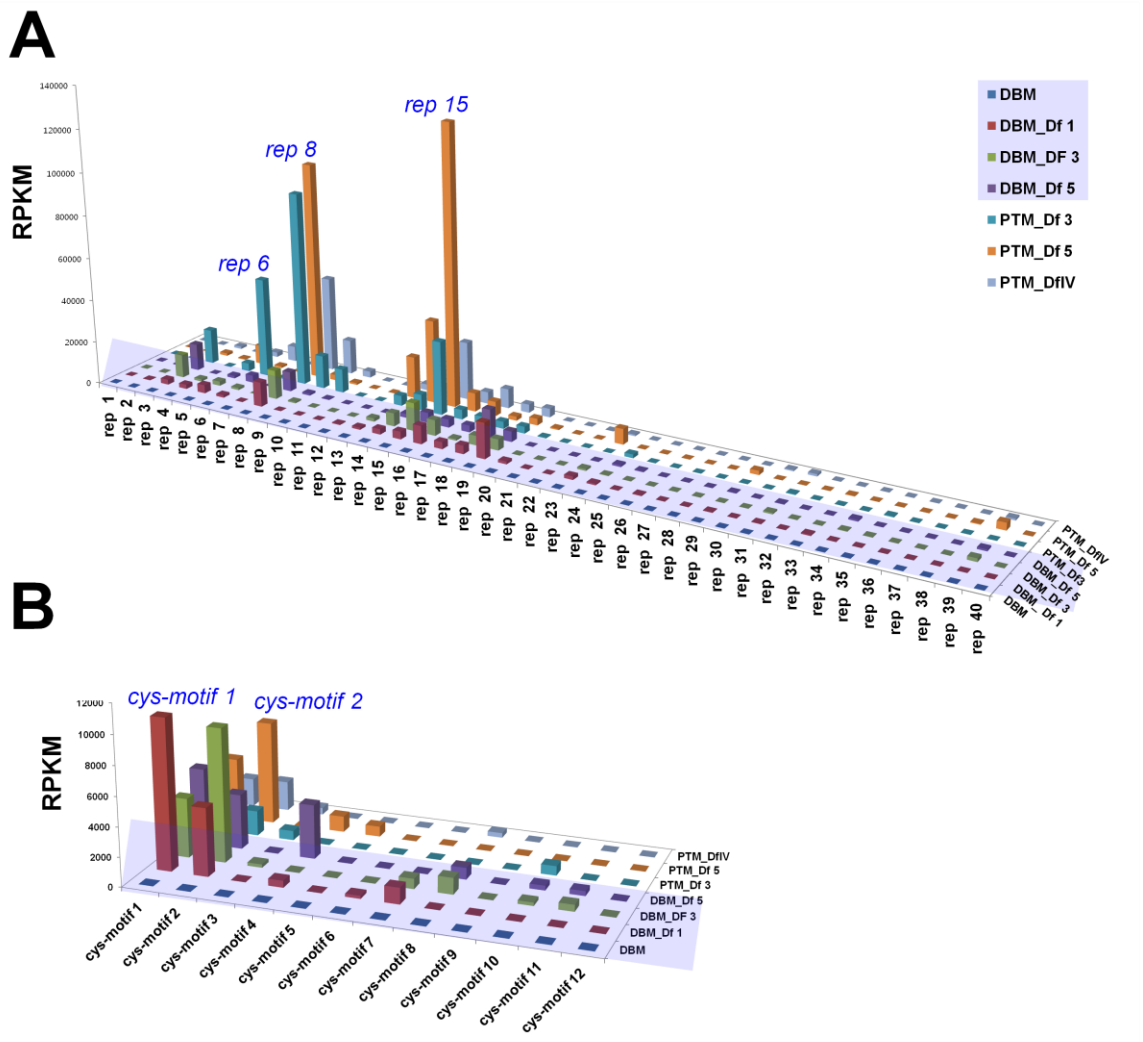


Fig. 23. Expression patterns of DfIV *rep* (A) and *cys-motif* (B) families in two lepidopteran hosts as determined by RNA-seq. Among the 40 *reps*, only ten *reps* significantly expressed (>10,000 in RPKM value, *rep*3, 6, 8, 9, 10, 13, 14, 15, 18 and 19). Generally, these *reps* were expressed to a high degree in PTM than DBM. This was especially true for the DfIV *rep* 15, 8 and 6. *Rep* 15, 8 and 6 were highly expressed in PTM especially, PTM_Df 3 and 5 samples.

Samples: DBM (unparasitized), DBM or PTM–Df 1, 3, 5 (one, three and five days after parasitized by *D. fenestrale*, respectively) and PTM–DfIV (unparasitized, virus injected by *D. fenestrale*).

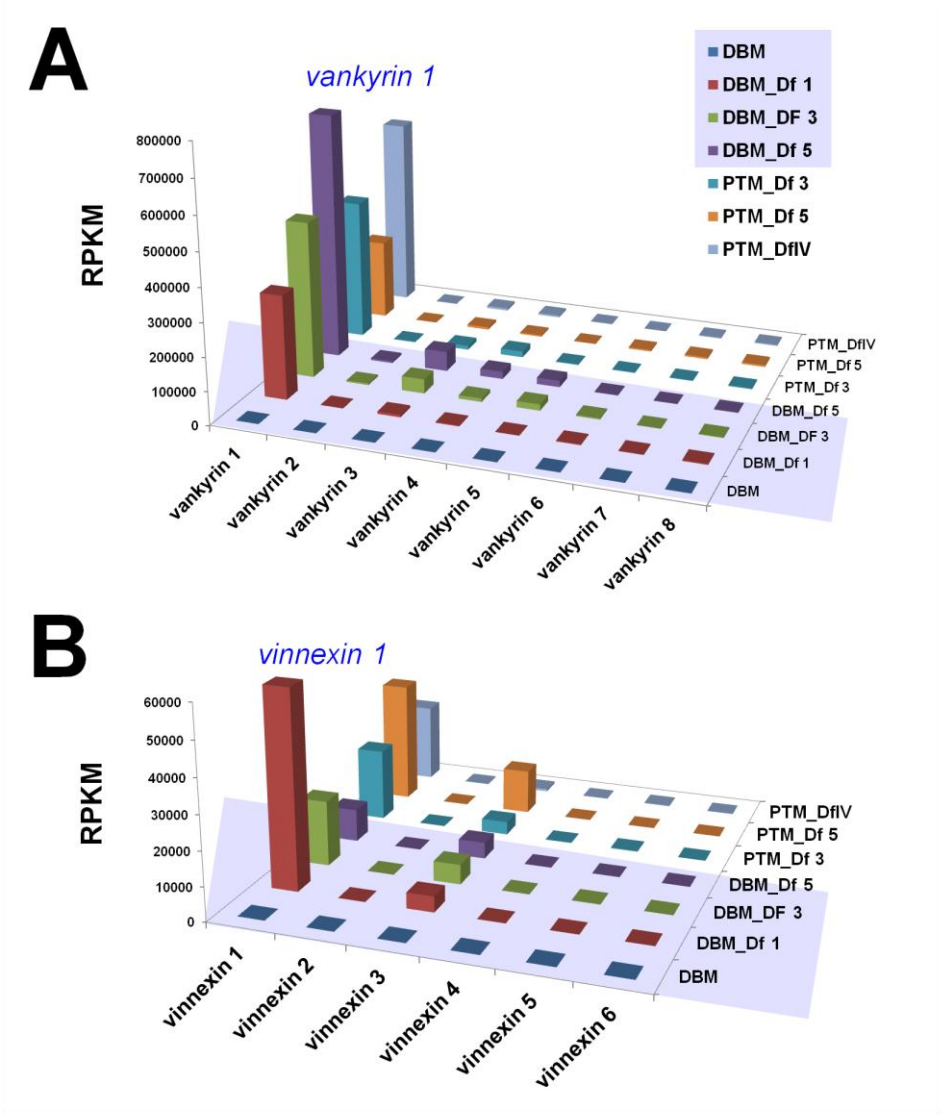


Fig. 24. Expression patterns of DfIV *vankyrin* (A) and *vinnexin* (B) families in two lepidopteran hosts as determined by RNA-seq. DfIV *vankyrin* 1 and *vinnexin* 1 were highly expressed in DBM and PTM. After parasitization, *vankyrin* became highly expressed while *vinnexin* was underexpressed in the DBM.

Samples: DBM (unparasitized), DBM or PTM–Df 1, 3, 5 (one, three and five days after parasitized by *D. fenestrale*, respectively) and PTM–DfIV (unparasitized, virus injected by *D. fenestrale*).

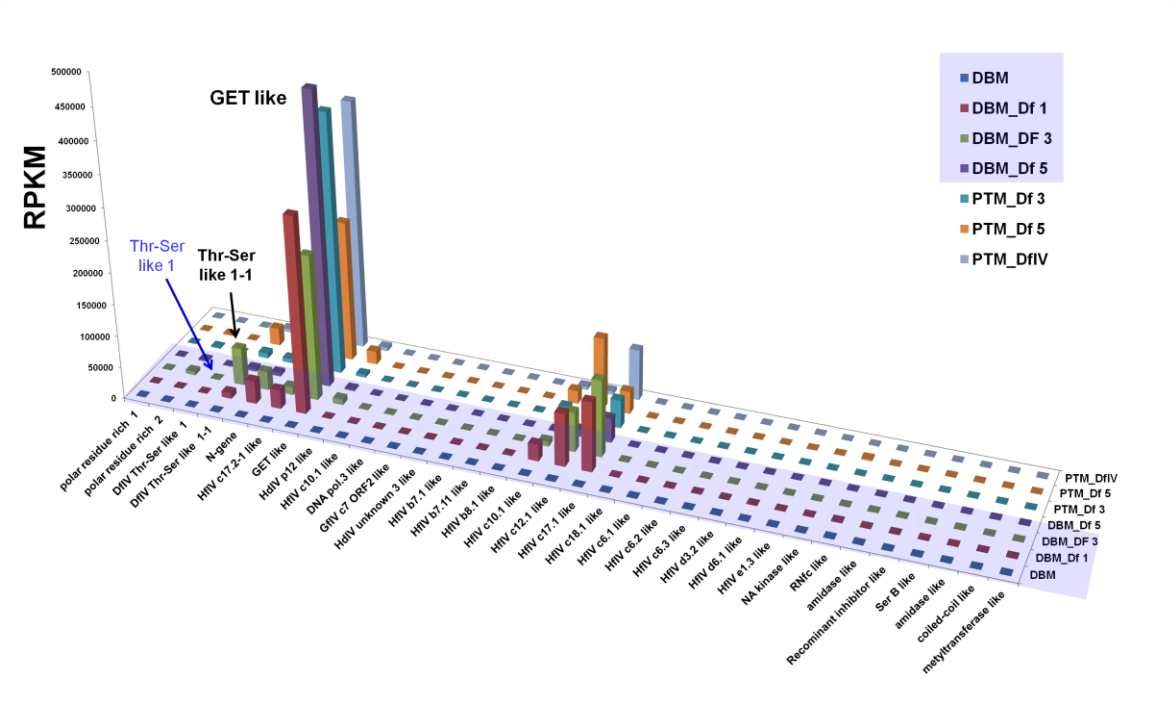


Fig. 25. DfIV unassigned gene expression patterns in two lepidopteran hosts as determined by RNA-seq. DfIV GET like gene was highly expressed in all parasitized and DfIV injected samples. Samples: DBM (unparasitized), DBM or PTM–Df 1, 3, 5 (one, three and five days after parasitized by *D. fenestrale*, respectively) and PTM–DfIV (unparasitized, virus injected by *D. fenestrale*).

3.3.2 qrtPCR-based expression analysis of DfIV genes

To validate the deep-sequencing-based DfIV gene expression pattern in lepidopteran hosts, qrtPCRs were conducted. Positive correlations were observed between qrtPCR and RNA-seq results. Various lepidopteran host samples with different developmental stages were used along with the DfIV gDNA as a reference. Lepidopteran larval stage was divided into six steps based on the parasitoid development. Sample name larva 1 to 6 means that *D. fenestrale* stages were: 1 – egg, 2 – 1st instar, 3 – 2nd instar, 4 – 3rd instar, 5 – early 4th instar and 6 – 4th instar. All larval samples were prepared from three treatment groups: unparasitized, parasitized and un-parasitized but DfIV injected by *D. fenestrale*. Additionally a pupa and adult samples were prepared from two treatments; unparasitized and un-parasitized but DfIV injected by *D. fenestrale*. Therefore, a total of 22 RNA samples from each host as well as the DfIV genomic DNA sample as a positive control were prepared. 73 genes were selected from the 99 DfIV genes (i.e., 38 *reps*, 8 *cys-motifs*, 7 *vankyrins*, 6 *vinnexins* and 24 other genes). These genes were selected based on either their nature of relative over-expression after parasitization (e.g., *vankyrin*) or their well/partially known expression patterns from other PDVs (e.g., *rep*, *vankyrin* and *vinnexin*) (Clavijo et al., 2011; Galibert et al., 2006; Turnbull and Webb, 2002).

All tested genes were not amplified in unparasitized samples except thr-ser like 1 and 1-1, particularly 1-1 gene in PTM. Therefore, these genes were excluded from the relative transcription analysis (Fig. 26). Generally, various genes were highly expressed in PTM, especially one day after parasitization. Some genes, such as HfIV c12.1 like, HdIV p12 like and GET like genes, exhibited much higher expression patterns in PTM than in DBM. Only some genes, *rep* 4 and 11, were more expressed in DBM at 1 day post-parasitization. When only the DfIV existed, , some genes, such as HfIV c12.1 like and GET like genes, were more expressed in PTM. DfIV genes were typically more expressed in PTM at the beginning of parasitization and then their expression diminished. In contrast, some genes, such as *rep* 11 and *vankyrin* 1, were continuously expressed throughout the

entire period of parasitization or some genes, such as *cys-motif 4* and HdIV p12 like etc., were highly expressed only in the late stage of parasitization.

Reps were differentially expressed in two hosts, particularly at 1 day post-parasitization. Most *reps* were expressed in PTM, but only some *reps* were expressed in DBM (Fig. 27). In PTM, the expression levels of *reps* decreased sequentially after parasitization except *rep 7*. Contrast to PTM, however, there was low clear correlation between the *rep* expression level and the timecourse of parasitizationin DBM. Only *rep 11* was highly expressed in DBM.

Cys-motif genes genes were also expressed in PTM, but only some *reps* were expressed in DBM at one day after parasitization. In PTM, *cys-motifs*' expression levels were sequentially decreased after parasitization but *cys-motifs* 1, 2, and 4 expressed, particularly *cys-motif* 4, were highly expressed in DBM at late larval stages.

Among the 7 *vankyrins*, *vankyrin 1* to *5* were continuously expressed over parasitization. In particular, *vankyrin 1* was mainly expressed in both hosts. There was some correlation between the *vankyrin* expression level and the parasitization time in PTM. However, there was no correlation in DBM.

Among the 6 *vinnexins*, *vinnexins 1* to *5* were constantly expressed in PTM. Especially *vinnexin 2* was mainly expressed in both hosts. *Vinnexin 2* was about 4 folds more expressed in PTM at 1 day post-parasitization than that of DBM.

Unassigned genes apart from the four main gene families were also expressed mainly in PTM at 1 day post-parasitization than in DBM (Fig. 28). Only HdIV p12 like gene was highly expressed in DBM at the late larval stages than that of PTM. From these results, it was clear that most of DfIV genes are predominantly expressed at the initial stage of parasitization in PTM, confirming the RNAseq data. On the other hands, few genes were expressed at the initial stage of parasitization in DBM and lower numbers of genes were expressed rather continuously or at the late stage of parasitization in DBM.

Relative DfIV gene copy numbers varied depending on gene amplification level (Fig. 29). The overall expression patterns of DfIV genes did not match to their own copy numbers. For example, *cys-motif* 1 and 2 were expressed almost the same level in all parasitized PTM samples. However, relative copy numbers of *cys-motif* 1 was much higher than that of *cys-motif* 2 (over 3,000). Nevertheless, NGS-based copy number estimation and that of qrtPCRs were showed some similarity each other. For example, the high copy segments 17 and 52 that carry *reps* 14 and 23 and *vinnexin* 2 were highly matched to respective expression level.

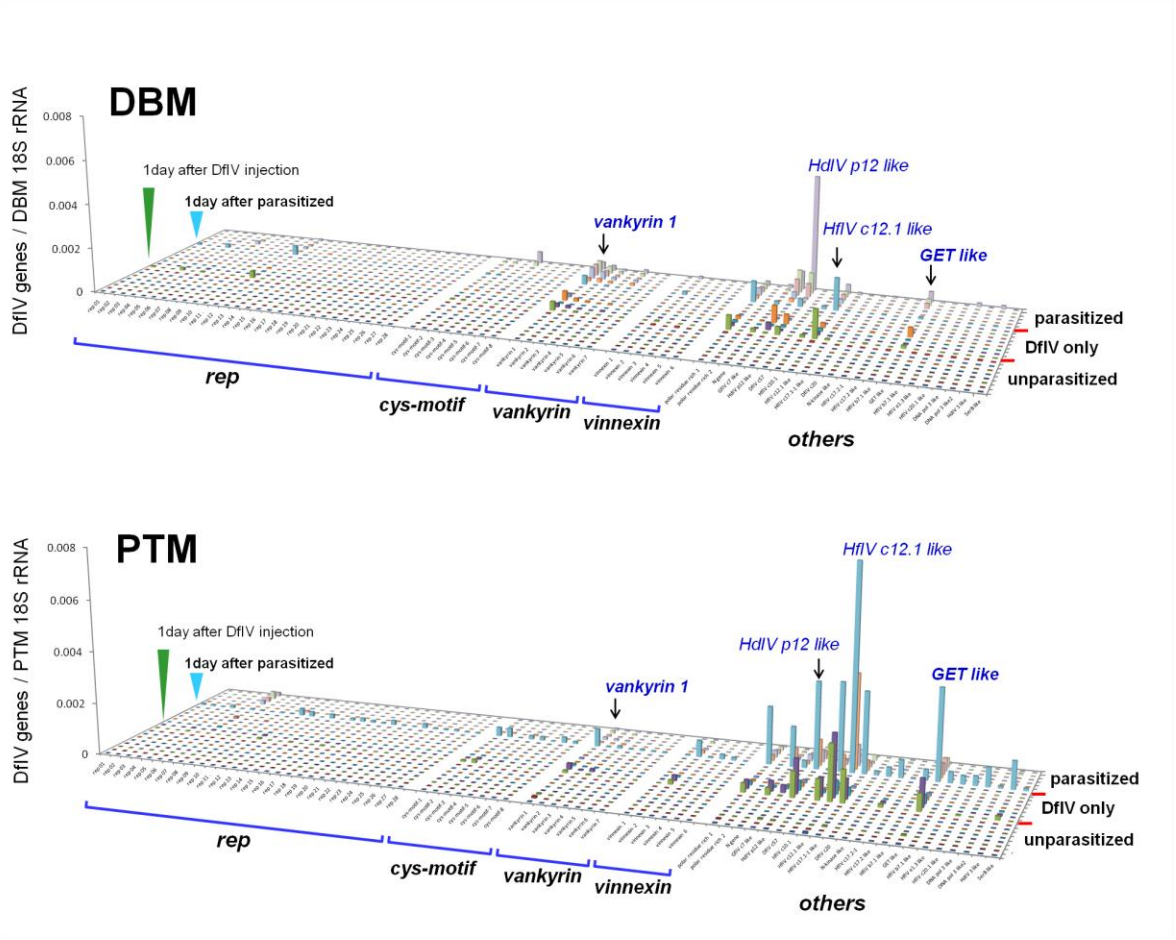


Fig. 26. qrtPCR results are shown in the relative transcript levels of DfIV genes. Three groups (unparasitized, unparasitized but DfIV injected by *D. fenestrale,* and parasitized) with six different laval samples (i.e., larvae 1 to 6 stand for the *D. fenestrale* developmental stages of egg, 1st, 2nd, 3rd, early and middle 4th instar, respectively, in each lepipdoptran host except the unparasitized group. Total RNA was extracted from these 44 samples, and qrtPCR reactions were run after cDNA synthesis. 73 genes were selected from the 99 DfIV (i.e., 28 *reps*, 8 *cyc-motifs*, 7 *vankyrins*, 6 *vinnexins* and 24 other un assigned genes). qrtPCRs were performed using the qrtPCR DELTAgene™ assays system (Fluidigm) with evagreen dye and qrtPCR primer sets (Table 5). Quantitative analysis was conducted by relative quantification method modified from the original concept of $2^{-\Delta\Delta Ct}$ methods.

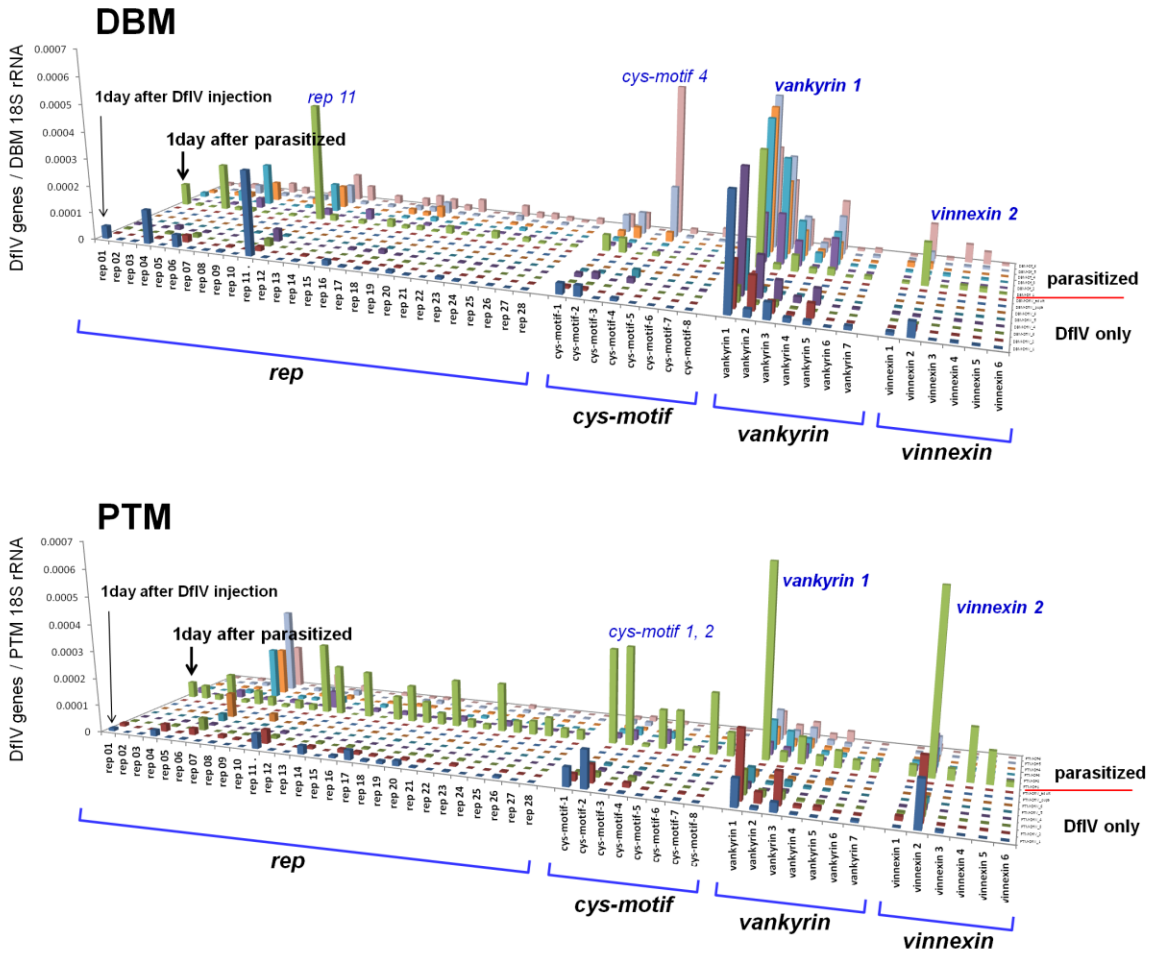


Fig. 27. qrtPCR results are shown in the relative transcript levels of DfIV *rep*, *cys-motif*, *vankyrin* and *vinnexin* gene families. Two groups (unparasitized but DfIV injected by *D. fenestrale*; and parasitized) with six different laval samples (i.e., larvae 1 to 6 stand for the *D. fenestrale* developmental stages of egg, 1st, 2nd, 3rd, early and middle 4th instar, respectively, in each lepipdoptran host except the unparasitized group. Initial expression levels of DfIV genes were higher in PTM than that of DBM such as *cys-motif 1* and *2*, *vankyrin 1* and *vinnexin 2*. Quantitative analysis was conducted by relative quantification method modified from the original concept of $2^{-\Delta\Delta Ct}$ methods.

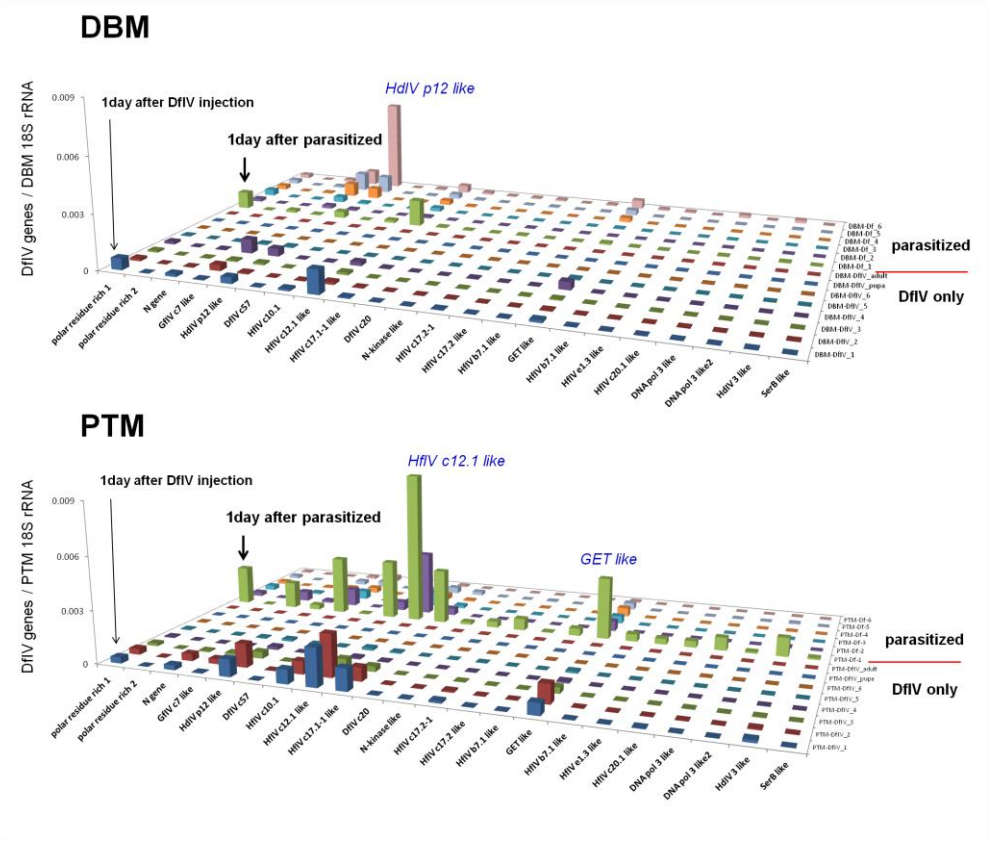


Fig. 28. qrtPCR results are shown in the relative transcript levels of unassigned DfIV genes. Two groups (unparasitized but DfIV injected by *D. fenestrale*; DfIV only and parasitized) with six different laval samples (i.e., larvae 1 to 6 stand for the *D. fenestrale* developmental stages of egg, 1st, 2nd, 3rd, early and middle 4th instar, respectively, in each lepipdoptran host except the unparasitized group. Initial expression levels of DfIV genes were higher in PTM than that of DBM such as HfIV c12.1 like and GET like. qrtPCRs were performed using the qrtPCR DELTAgene™ assays system (Fluidigm) with evagreen dye and qrtPCR primer sets (Table 5). Quantitative analysis was conducted by relative quantification method modified from the original concept of $2^{-\Delta\Delta Ct}$ methods.

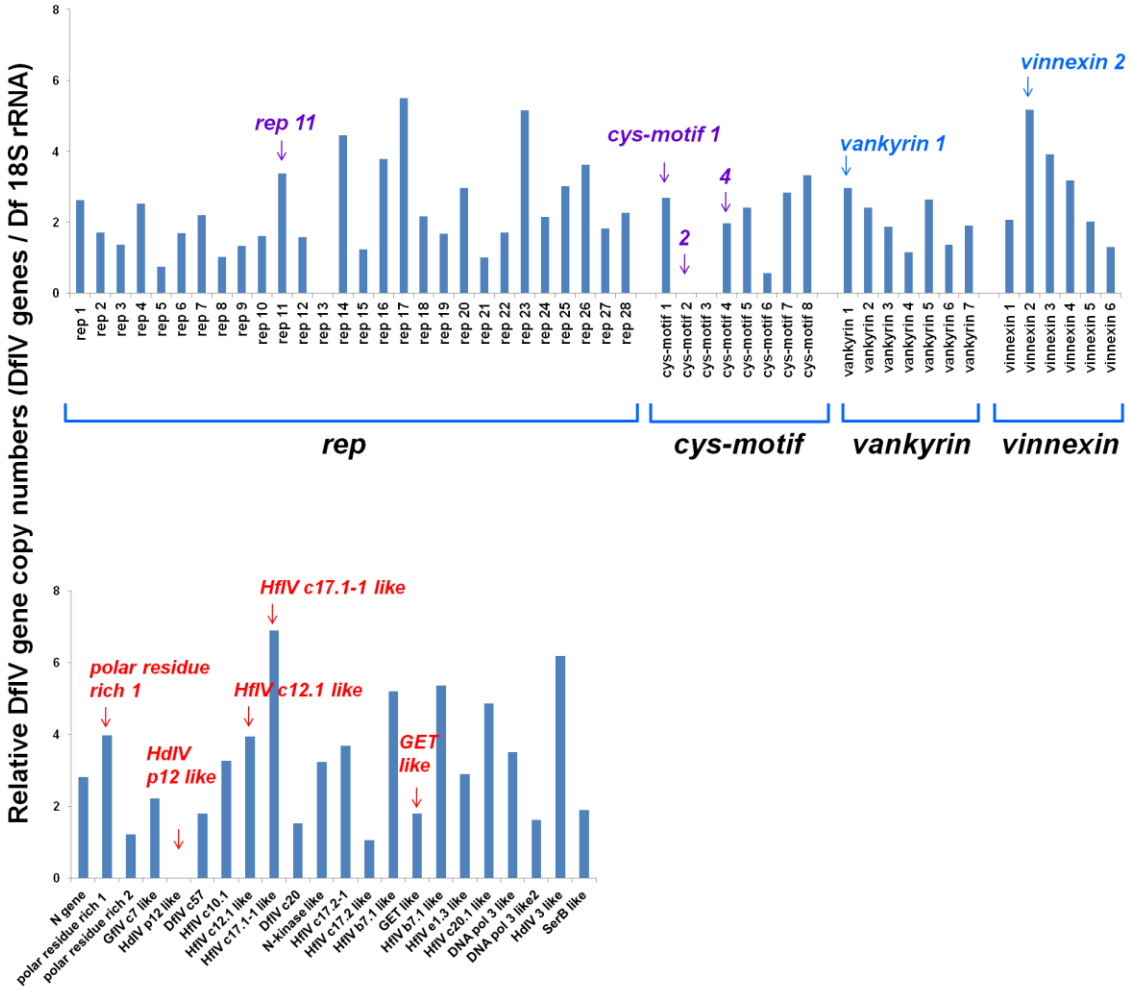


Fig. 29. Copy numbers of the DfIV genome segments that contain the DfIV genes examined in this study as estimated by qrtPCR. DfIV genome segments' copy numbers were relatively calculated to the *cys-motif* 2 gene amplification level. Gene amplification levels were calculated by the relative quantification method modified from the original concept of $2^{-\Delta\Delta Ct}$ methods with *D. fenestrale* (Df) 18S rRNA which used as a reference. All tested genes were amplified in DfIV gDNA. Colored named genes means that highly expressed in PTM and/or DBM (purple, over 0.003; blue, over 0.0006 and red, over 0.002 relative transcript levels, respectively).

4. Discussion

Most parasitoid species identified as generalists are actually complexes of closely related and relatively specialized taxa (Stireman, 2005). *D. fenestrale* (Hymenoptera: Ichneumonidae: Campopleginae) appears to be a true generalist by parasitizing the PTM larvae as well as DBM as hosts in both open field and laboratory condition (Choi et al., 2013; Kim et al., 2012). Even though *D. fenestrale* can parasitize both PTM and DBM, they are individually grouped from comprehensive phylogenetic tree in Lepidoptera (Heikkila et al., 2012; Mutanen et al., 2010). PTM and DBM were calssfied in Ditrysia and divided in superfamily level, Gelechioidea and Yponomeutoidea, respectively (Kristensen et al., 2007). Evolutionary studies in Tachinidae (the most species rich group of parasitic fly, Diptera) conclude that the evolutionary flow in host ranges showed generalist to specialist (Stireman, 2005). *D. fenestrale* parasitize both lepidopteran hosts, but their parasitic rate (91.7 % in PTM and 73.3 % in DBM) and survival rate (83.3 % in PTM and 46.7 % in DBM) were different two hosts. Here, I have two questions. First, how *D. fenestrale* could be adopted in different eviromental condition? Second, what is the main factor that makes the survival of parasitoids different when they develop in two lepidopteran hosts?

First, *D. fenestrale* could normally grow in two different hosts but the host larval period after parasitization was extended for 1-2 and 2-3 days in PTM and DBM, respectively. Therefore, this finding indicates that *D. fenestrale* can regulate the developmental period of lepidopteran host for its own survival and their maturation. These host development regulations were controlled by Juvenile hormone (JH) synthesis from parasitoid (Li et al., 2003; Schafellner et al., 2004) and/or JH esterase (JHE) overexpression from PDV (Cusson et al., 2000). Untill now, I did not analyzed JH and JHE concentration and activity, but probably JH and/or JHE contribute to control the lepidopteran host development for parsitoid.

Second, the host preference and parasitoid survival rate is the result of complicated mechanism. There cases were reported in *C. sonorensis* with CsIV (Cui et al., 2000; Webb and Cui, 1998). Host cellular immune responses to parasitoid eggs appear to be important factor determining the level of success of parasitism and restricting host range. For example, generalist *C. sonorensis* parasitizes as many as 27 different lepidopteran species (Lingren et al., 1970). However, the level of success for parasitism varies among host species. *C. sonorensis* adults oviposit in lepidopteran larvae of several species including those in which parasitoid development is not successful. Hosts that do not support their development are considered non-permissive to parasitism. The molecular basis for successful parasitism or determination of host-range for most parasitoids is not well understood. However, some cases were reported that PDVs participate in host range determination. The one of the *cys-rich* CsIV VHv1.4 was differentially expressed in their lepidopteran hosts. Successful parasitism of *C. sonorensis* depends on the CsIV VHv1.4 expression level and durability (Cui et al., 2000). Therefore, I focused on the PDV gene expression patterens. To identify the relationship between the survival rate and host preference of *D. fenestrale* and the DfIV expression patterns in two lepidopteran hosts, RNA-seqs were conducted using samples of various hosts' conditions. Based on the expression quantification methods (Mortazavi et al., 2008), DfIV gene expression levels were directly compared in all tested conditions between PTM and DBM. Among these genes, some *reps* were highly expressed in only PTM. However, much information on DfIV expression patterns in each host could not be obtained because of the loss of three samples during the procedure of RNA-seq. As a result, it was not possible to analyze comparatively the whole sets of transcripts. However, comprehensive DfIV gene expression patterns could be estimated.

To validate the RNA-seq based DfIV gene expression pattern in lepidopteran hosts, qrtPCRs were conducted. Samples were designed not only to validate the RNA-seq data but also to get integrated information in particular post parasitization time lapse. Therefore, a total of 22 RNA samples were prepared from each host as well as the DfIV gDNA sample was also prepared as a positive control. As described above, RNA-seq results could be compared under all conditions but

only relative comparison between two hosts could be possible. Although there were existed some disagreement between RNA-seq data and qrt-PCR results, there was positive correlation observed in remaining data set between RNA-seq and qrtPCR results. Most DfIV genes were more expressed in PTM than DBM especially within a day after parasitized. These initial responses were very important to determine the success or fail of parasitism (Webb, 1998.).

Taken together, most of DfIV genes more expressed in PTM and these expressed genes contribute to increase the survival rate. This is one of the evidence that they have co-relationship between parasitoids and PDVs. Additionally, there were no correlation between copy numbers and expression level in lepidopteran hosts. PDV gene expression level also can be controlled by their promoter (Choi et al., 2009a; Choi et al., 2009b; Soldevila and Webb, 1996). These DfIV promoters can be applicable to various research fields.

Conclusion

Overall, this study provides the first comprehensive analysis of new PDV, DfIV and their expression patterns in two lepidopteran hosts. DfIV has 65 genome segments and the entire DfIV genome (247,191 bp) was sequenced and annotated. Ninety nine genes were predicted and, based on these genes' phylogenetic relationship; DfIV was categorized as a typical IV. *D. fenestrale* is able to parasitize two lepidopteran caterpillars, the PTM and DBM. Their oviposition and survival rate results showed that *D. fenestrale* preferred PTM to DBM as host. Moreover, DfIV genes were highly expressed in PTM than that of DBM, particularly at initial point after parasitized. In addition, some DfIV genes have differential expression patterns in their two lepidopteran hosts during the time course of parasitization. Therefore, I concluded that DfIV over-expression and/or initial expression in PTM could contribute to the increase of the parasitoid survival.

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Supplementary table 1.Size, GC contents and BLAST results of DfIV genomic segments

Segment	size (bp)	GC %	Description of BLAST results	Accession	E value	Max identity
1	5240	41.0	HfIV, segment C19	AB291194.1	6.00E-148	86%
2	4843	42.5	HfIV, segment D10	AB291204.1	0	80%
3	5552	45.4	HfIV, segment C8	AB291186.1	0	79%
4	6054	43.5	HfIV, segment D8	AB291202.1	0	85%
5	5832	43.1	HfIV, segment D10	AB291204.1	0	87%
6	5997	42.2	HfIV, segment C4	AB291182.1	0	80%
7	5528	42.5	HfIV, segment D9	AB291203.1	0%	79%
8	4784	42.4	HfIV, segment D10	AB291204.1	0	80%
9	4930	42.4	HfIV, segment D9	AB291203.1	0	85%
10	3116	42.0	HfIV, segment A3	AB291166.1	3.00E-136	81%
11	6602	42.5	HfIV, segment D11	AB291205.1	0	86%
12	4153	44.9	HfIV, segment C8	AB291186.1	0	81%
13	5198	42.7	HfIV, segment D7	AB291201.1	0	78%
14	3438	43.5	HfIV, segment B4	AB291169.1	0	82%
15	3884	44.7	HfIV, segment C12	AY556384.1	0	90%
16	4406	44.8	HfIV, segment D6	AB291200.1	0	79%
17	5185	43.1	HfIV, segment D1	AB291196.1	0	96%
18	3243	42.5	HfIV, segment B1	AY935249.1	0	78%
19	4448	42.9	HfIV, segment C10	AY577429.1	1.00E-117	85%
20	4939	44.1	HfIV, segment C6	AB291184.1	3.00E-139	85%
21	5753	42.7	HfIV, segment C3	AB291181.1	0	82%
22	4055	44.0	HfIV, segment C9	AB291187.1	0	79%
23	4428	44.4	HfIV, segment B16	AB291178.1	0	84%
24	3165	42.8	HfIV, segment D9	AB291203.1	0	77%
25	4074	42.8	HfIV, segment C14	AB291190.1	3.00E-113	80%
26	4738	43.0	HfIV, segment D4	AB291199.1	0	84%
27	3722	42.5	TrIV, segment C6	AB291146.1	1.00E-98	69%
28	4752	43.3	HfIV, segment C18	AB291193.1	0	77%
29	2662	43.4	HfIV, segment B12	AB291174.1	0	78%
30	4511	41.8	HfIV, segment C16	AY547319.1	0	85%
31	2981	44.2	HfIV, segment C5	AB291183.1	0	76%
32	2881	44.1	HfIV, segment B14	AB291176.1	3.00E-142	80%
33	3547	43.6	HfIV, segment B5	AB291170.1	0	81%

Supplementary table 1 (continued)

Segment	size (bp)	GC %	Description of BLAST results	Accession	E value	Max identity
34	4547	44.3	HfIV, segment E2	AB291208.1	0	86%
35	4289	42.2	HfIV, segment C7	AB291185.1	0	80%
36	2459	41.6	HfIV, segment C16	AY547319.1	3.00E-136	85%
37	3744	42.3	HfIV, segment B7	AY563518.1	0	86%
38	4125	43.8	HfIV, segment C2	AY570799.1	2.00E-153	84%
39	2405	43.0	HfIV, segment B12	AB291174.1	0	84%
40	2980	40.0	HfIV, segment C4	AB291182.1	0.001	74%
41	2392	42.6	HfIV, segment D11	AB291205.1	1.00E-172	87%
42	4794	42.8	HfIV, segment D11	AB291205.1	0.00E+00	89%
43	3540	45.9	HfIV, segment E1	AB291207.1	1.00E-136	87%
44	4158	44.4	HfIV, segment B17	AY577428.1	0	86%
45	3243	44.9	HfIV, segment B11	AY570798.1	7.00E-126	77%
46	2036	43.9	HfIV, segment C17	AB291192.1	0	77%
47	3337	43.4	HdIV, segment11	AF364056.1	0	76%
48	4504	43.7	HfIV, segment B17	AY577428.1	0	86%
49	4691	44.3	HfIV, segment C18	AB291193.1	0	81%
50	1919	43.0	HfIV, segment C11	AB291188.1	4.00E-170	82%
51	2613	42.6	HfIV, segment D12	AB291206.1	4.00E-96	89%
52	4510	41.8	HfIV, segment C16	AY547319.1	0	85%
53	3320	42.6	HfIV, segment B14	AB291176.1	1.00E-110	81%
54	1749	45.5	HfIV, segment C1	AB291180.1	4.00E-113	76%
55	3612	44.4	HfIV, segment C6	AB291184.1	5.00E-78	85%
56	2300	44.3	HfIV, segment B17	AY577428.1	0	86%
57	1511	45.5	HfIV, segment C1	AB291180.1	2.00E-172	77%
58	3539	43.4	HfIV, segment B8	AY597814.1	0	82%
59	2573	45.5	HfIV, segment C1	AB291180.1	5.00E-127	76%
60	1714	42.1	HfIV, segment B17	AY577428.1	0	85%
61	3745	42.2	HfIV, segment B7	AY563518.1	0	82%
62	1880	45.2	HfIV, segment D9	AB291203.1	0	85%
63	1893	42.6	HfIV, segment B13	AB291175.1	2.00E-131	73%
64	2210	44.0	HfIV, segment C17	AB291192.1	2.00E-162	80%
65	1426	42.9	HfIV, segment D9	AB291203.1	0	81%
Total	**247,191**					

Supplementary table 2. Predicted genes in DfIV circular genome segments

Segment (size, bp)	DfIV genes	Frame	from [a]	to	Length (a.a)	Description	Accession
1 (5240)	cys-motif 3	2	3326	3607	94	cysteine motif c19.1 [HfIV]	YP_001031361.1
	cys-motif 1	-1	4953	5216	88	A'Hv0.8 cys-motif [CsIV]	AAO43443.1
	Hc1-1 [b]	-3	1570	1854	95	No hit	
	Hc1-2	3	4098	4340	81	No hit	
	Hc1-3	1	1270	1497	76	No hit	
	Hc1-4	1	3895	4107	71	No hit	
	Hc1-5	-1	2715	2921	69	No hit	
2 (4843)	rep 8 [c]	-2	2593	3294	234	c7-1.1 [TrIV]	BAF45598.1
	rep7	-3	4437	352	253	c7-1.1 [TrIV]	BAF45598.1
	Hc2-1	3	2742	3005	88	No hit	
	Hc2-2	-2	4189	4437	83	No hit	
	Hc2-3	3	270	518	83	No hit	
	Hc2-4	-1	1337	1564	76	d3.2 [HfIV]	YP_001031313.1
	Hc2-5	-3	1074	1292	73	No hit	
3 (5552)	GET like	2	1766	2596	277	protein piccolo [*Ovis aries*]	XP_004008269.1
	rep 28	-3	3211	3870	220	f3.2 [TrIV]	BAF45626.1
	Hc3-1	-1	1752	2162	137	No hit	
	Hc3-2	2	3773	4048	92	No hit	
	Hc3-3	-2	3161	3403	81	intraflagellar transport protein 172 homolog [*Papio anubis*]	XP_003908478.1
	Hc3-4	3	2097	2333	79	No hit	
	HdIV [d] unknown like	-2	4580	4807	76	unknown [HdIV]	AAO33350.1
	Hc3-5	1	1276	1482	69	No hit	
	Hc3-6	3	183	389	69	No hit	
	Hc3-7	-1	822	1025	68	No hit	
4 (6054)	vankyrin 6	2	2840	3421	194	vankyrin 2 [HdIV]	AFH35118.1
	vankyrin 4	1	4924	5433	170	vankyrin 5 [HdIV]	AFH35119.1
	vankyrin 8	1	1609	2118	170	vankyrin 2 [HdIV]	AFH35116.1
	vankyrin 7	1	325	831	169	vankyrin 4 [HdIV]	AFH35118.1
	vankyrin 3	1	3541	4041	167	vankyrin 1 [HdIV]	AFH35115.1
	Hc4-1	1	2476	2853	126	No hit	
	Hc4-2	-1	5560	5901	114	No hit	
	Hc4-3	2	4517	4795	93	No hit	
	Hc4-4	-1	1189	1461	91	No hit	
	Hc4-5	-2	837	1091	85	No hit	
	Hc4-6	-3	1352	1594	81	No hit	
	Hc4-7	2	5540	5755	72	No hit	
	Hc4-8	3	933	1148	72	No hit	
	Hc4-9	3	2220	2432	71	No hit	

[a] ORF start point, [b] Hypothetical protein, [c, d] some gene name and PDV names were used abbreviation, documented in bottom of this table

ORF finder was used and ORF length was limited over 200bp.

Supplementary table 2 (continued)

Segment (size, bp)	DfIV genes	Frame	from	to	Length (a.a)	Description	Accession
5 (5832)	rep 22	-2	603	1415	271	c7-1.1 [TrIV]	BAF45598.1
	rep 10	-2	4602	5315	238	repeat element protein-d10.1 [HfIV]	YP_001031335.1
	Hc5-1	-1	757	1062	102	No hit	
	Hc5-2	-1	5395	5640	82	No hit	
	Hc5-3	3	3690	3905	72	No hit	
	Hc5-4	3	3327	3533	69	No hit	
6 (5997)	cys-motif 4	-3	1277	1657	127	cysteine motif gene-c4.1 [HfIV]	YP_001031276.1
	Hc6-1	1	400	663	88	No hit	
	Hc6-2	2	2708	2959	84	No hit	
	Amidase	-1	250	501	84	amidase [*Rhodococcus erythropolis*]	AEX32473.1
	cys-motif 9	-3	5153	5398	82	cysteine motif gene-c19.1 [HfIV]	YP_001031361.1
	Hc6-3	-1	5659	5889	77	No hit	
	Hc6-4	-3	302	508	69	No hit	
7 (5528)	cys-motif 8	3	4296	4880	195	cysteine motif gene-d9.1 [HfIV]	YP_001031331.1
	cys-motif 10	3	63	470	136	cysteine motif gene-d9.1 [HfIV]	YP_001031331.1
	cys-motif 11	1	1171	1575	135	cysteine motif gene-d9.2 [HfIV]	YP_001031333.1
	Hc7-1	-1	4305	4598	98	No hit	
	Hc7-2	3	3891	4175	95	No hit	
	Hc7-3	2	2345	2584	80	No hit	
	Hc7-4	3	2946	3170	75	No hit	
8 (4784)	rep 40	-1	2010	2882	291	repeat element protein-d10.3 [HfIV]	YP_001031337.1
	rep 9	-1	123	839	239	c7-1.1 [TrIV]	BAF45598.1
	HfIV d3.2 like	-3	3520	3909	130	d3.2 [HfIV]	YP_001031313.1
	Hc8-1	-3	2098	2466	123	No hit	
	Hc8-2	-2	3188	3511	108	No hit	
	Hc8-3	2	152	472	107	No hit	
	Hc8-4	1	3370	3684	105	No hit	
	Hc8-5	-1	3795	4058	88	No hit	
	Hc8-6	2	3770	4000	77	No hit	
9 (4930)	cys-motif 5	2	4346	4929	195	cysteine motif gene-d9.1 [HfIV]	YP_001031331.1
	Hc9-1	-3	3921	4244	108	No hit	
	Hc9-2	-1	1655	1942	96	No hit	
	Coiled-coil	-2	2335	2613	93	coiled-coil domain-containing protein [*Nasonia vitripennis*]	XP_003428001.1
	cys-motif 12	3	753	1019	89	cysteine motif gene-d9.2 [HfIV]	YP_001031333.1
	Hc9-3	-2	4534	4794	87	No hit	
	Hc9-4	2	3149	3409	87	No hit	
	Hc9-5	2	2816	3067	84	No hit	
	Hc9-6	3	1812	2036	75	No hit	
	Hc9-7	-2	1948	2163	72	No hit	
	Hc9-8	-1	2	217	72	No hit	

Supplementary table 2 (continued)

Segment (size, bp)	DfIV genes	Frame	from	to	Length (a.a)	Description	Accession
10 (3116)	Hc10-1	2	302	571	90	No hit	
	Hc10-2	-3	2344	2610	89	No hit	
	HfIV a3.1 like	3	516	782	89	a3.1 [HfIV]	YP_001031244.1
	Hc10-3	-2	488	691	68	No hit	
11 (6602)	rep 16	3	963	1721	253	c7-1.1 [TrIV]	BAF45598.1
	rep 29	2	2768	3511	248	c7-1.1 [TrIV]	BAF45598.1
	rep 17	-1	4596	5201	202	c7-1.1 [TrIV]	BAF45598.1
	rep 26-1	1	19	441	141	c7-1.1 [TrIV]	BAF45598.1
	Hc11-1	3	3222	3473	84	No hit	
	Hc11-2	-2	464	712	83	No hit	
	Hc11-3	-3	58	297	80	No hit	
	Hc11-4	-2	809	1033	75	No hit	
	Hc11-5	1	4507	4728	74	No hit	
	Hc11-6	-2	3164	3367	68	No hit	
12 (40153)	Thr-ser like 1	3	2181	2687	169	thr-ser protein [HdIV]	AAO33571.1
	Thr-ser like 1-1	3	1675	2687	148	thr-ser protein [HdIV]	AAO33571.1
	Hc12-1	-1	827	1078	84	No hit	
	Hc12-2	1	1573	1821	83	No hit	
	Hc12-3	-1	3197	3424	76	No hit	
	Hc12-4	-3	2889	3095	69	No hit	
	Hc12-5	-1	1577	1783	69	No hit	
	Hc12-6	-3	615	821	69	No hit	
	Hc12-7	3	135	338	68	No hit	
13 (5198)	rep 24	-1	1048	1812	255	c7-1.1 [TrIV]	BAF45598.1
	rep 30	-3	4013	4744	244	repeat element protein-d7.2 [HfIV]	YP_001031325.1
	rep 31	-2	2841	3557	239	repeat element protein-d7.3 [HfIV]	YP_001031326.1
	Hc13-1	1	4699	5109	137	No hit	
	Hc13-2	-1	3433	3777	115	No hit	
	TrIV c289.2 like	-2	1278	1607	110	c289.2 [TrIV]	BAF45770.1
	Hc12-3	3	2160	2372	71	No hit	
14 (3438)	rep 32	2	1133	1768	212	repeat element protein-b4.1 [HfIV]	YP_001031251.1
	rep 27	1	2926	138	217	c7-1.1 [TrIV]	BAF45598.1
	Hc14-1	3	552	764	71	No hit	
	Hc14-2	2	3014	3223	70	No hit	
15 (3884)	HfIV c12.1 like	1	2323	3639	439	c12.1 [HfIV]	YP_001031225.1
	Hc15-1	2	458	769	104	No hit	
	Hc15-2	-2	2630	2866	79	No hit	
	Hc15-3	-1	3585	3800	72	No hit	
	Hc15-4	2	1589	1789	67	No hit	

Supplementary table 2 (continued)

Segment (size, bp)	DfIV genes	Frame	from	to	Length (a.a)	Description	Accession
16 (4406)	rep 33	2	188	925	246	repeat element protein-b15.1 [HfIV]	YP_001031263.1
	rep 25	-2	2021	2650	210	c7-1.1 [TrIV]	BAF45598.1
	Hc16-1	2	2636	2950	105	No hit	
	HfIV d6.1 like	2	2126	2434	103	d6.1 [HfIV]	YP_001031321.1
	Hc16-2	-3	610	825	72	No hit	
	Hc16-3	-2	4199	4405	69	No hit	
17 (5185)	rep 14	3	891	1460	190	repeat element protein-d2.1 [HfIV]	YP_001031304.1
	rep 23	3	2415	2978	188	c7-1.1 [TrIV]	BAF45598.1
	Hc17-1	-1	3215	3490	92	No hit	
	Hc17-2	-3	3702	3953	84	No hit	
	Hc17-3	-3	3294	3506	71	No hit	
18 (3243)	vankyrin 1	-3	2288	2794	169	vankyrin-b1 [HfIV]	AAX24120
	Hc18-1	2	104	325	74	No hit	
	Hc18-2	-1	625	828	68	No hit	
	Hc18-3	3	393	596	68	No hit	
19 (4448)	HfIV c10.1 like (RNA pol.)	3	1266	2597	444	c10.1 [HfIV]	YP_001031234.1
	Hc19-1	1	2377	2685	103	No hit	
	Hc19-2	-2	269	538	90	No hit	
	Hc19-3	1	1600	1830	77	No hit	
	Hc19-4	1	2872	3087	72	No hit	
	Hc19-5	3	912	1112	67	No hit	
20 (4939)	HfIV c6.3 like	-1	3875	4480	202	c6.3 [HfIV]	YP_001031282.1
	unknown 8	1	682	1110	143	No hit	
	HfIV c6.2 like	-3	1695	1991	99	c6.2 [HfIV]	YP_001031281.1
	Hc20-1	-2	2368	2637	90	No hit	
	Hc20-2	-2	445	693	83	No hit	
	Hc20-3	1	205	453	83	No hit	
	Hc20-4	2	1178	1408	77	No hit	
21 (5753)	vinnexin 3	1	1087	2169	361	innexin Vnx-c16 [HfIV]	YP_001031223.1
	rep 34	2	3416	4102	229	repeat element protein-c3.1 [HfIV]	YP_001031274.1
	rep 35	1	5368	304	230	repeat element protein-c3.1 [HfIV]	YP_001031274.1
	Hc21-1	3	3207	3479	91	No hit	
	Hc21-2	-2	2582	2797	72	No hit	
	Hc21-3	3	1797	2009	71	No hit	
	Hc21-4	2	4781	4990	70	No hit	
	Hc21-5	-3	5527	5730	68	No hit	
	Hc21-6	3	3774	3974	67	No hit	
22 (4055)	Unknown1 (Ngene)	-3	91	1482	464	N gene-c9.1 [HfIV]	YP_001031285.1
	Hc21-1	2	1208	1507	100	No hit	
	metyltransferase like	2	173	430	86	putative methyltransferase [*Serratia plymuthica* PRI-2C]	ZP_10110886.1
	Hc21-2	3	2760	2990	77	No hit	

Supplementary table 2 (continued)

Segment (size, bp)	DfIV genes	Frame	from	to	Length (a.a)	Description	Accession
23 (4428)	NA kinase like	1	3472	3993	174	N-acetylmannosamine kinase [*Haemophilus influenzae* R3021]	ZP_01797053.1
	Hc23-1	-1	3607	3978	124	No hit	
	Hc23-2	-2	375	746	124	No hit	
	Hc23-3	-2	3501	3818	106	No hit	
	Hc23-4	1	745	957	71	No hit	
	Hc23-5	-3	3965	4171	69	No hit	
24 (3165)	cys-motif 7	1	1360	2166	269	cysteine motif gene-d9.1 [HfIV]	YP_001031331.1
	Hc24-1	3	324	611	96	No hit	
	Hc24-2	-2	207	479	91	No hit	
	Hc24-3	3	2751	3020	90	No hit	
25 (4074)	Hc25-1	-2	792	1064	91	No hit	
	Hc25-2	1	1384	1641	86	No hit	
	Hc25-3	3	2367	2609	81	No hit	
	Hc25-4	-2	2019	2261	81	No hit	
	Hc25-5	3	2826	3056	77	No hit	
	Hc25-6	1	2224	2439	72	No hit	
	Hc25-7	2	386	595	70	No hit	
	Hc25-8	3	1611	1811	67	No hit	
	Hc25-9	-3	1340	1540	67	No hit	
26 (4738)	rep 6	2	317	1063	249	repeat element protein-d4.2 [HfIV]	YP_001031316.1
	rep 3	2	3320	3991	224	repeat element protein-d4.1 [HfIV]	YP_001031315.1
	Hc26-1	3	957	1307	117	No hit	
	Hc26-2	-2	1291	1602	104	No hit	
	Hc26-3	-2	3292	3498	69	No hit	
	Hc26-4	-1	1118	1324	69	No hit	
	Hc26-5	-1	422	628	69	No hit	
	Hc26-6	-3	2709	2912	68	No hit	
27 (3722)	vinnexin 5	2	1067	2260	398	d4.1 [TrIV]	BAF45609.1
	Hc27-1	-3	871	1185	105	No hit	
	Hc27-2	2	131	409	93	No hit	
	Hc27-3	-2	593	862	90	No hit	
	Hc27-4	-1	3186	3434	83	No hit	
	Hc27-5	-3	2302	2523	74	No hit	
	Hc27-6	3	3465	3668	68	No hit	
28 (4752)	rep 11	-3	2186	2893	236	repeat element protein-c18.1 [HfIV]	YP_001031294.1
	rep 1	-1	3976	4653	226	c7-1.1 [TrIV]	BAF45598.1
	rep 4	-3	947	1411	155	f3.2 [TrIV]	BAF45626.1
	HfIV d6.1 like	1	4123	4383	87	d6.1 [HfIV]	YP_001031321.1
	HfIV c18.1 like	1	1735	1986	84	c18.1 [HfIV]	YP_001031295.1
	Hc28-1	-1	2251	2472	74	No hit	
	Hc28-2	2	4265	4474	70	No hit	
	Hc28-3	-3	2948	3154	69	No hit	

Supplementary table 2 (continued)

Segment (size, bp)	DfIV genes	Frame	from	to	Length (a.a)	Description	Accession
29 (2662)	rep 20	-3	600	1307	236	f3.2 [TrIV]	BAF45626.1
	Hc29-1	2	1571	1837	89	No hit	
30 (4511)	vinnexin 2	3	9	992	328	innexin Vnx-g1 [CsIV]	AAO45829.1
	Hc30-1	-3	3949	4269	107	No hit	
	Hc30-2	-2	2321	2578	86	No hit	
	Hc30-3	-2	2810	3064	85	No hit	
	Hc30-4	-1	3471	3683	71	No hit	
	Hc30-5	1	574	783	70	No hit	
	Hc30-6	1	2872	3078	69	No hit	
	Hc30-7	-1	2382	2582	67	No hit	
	Hc30-8	-1	306	506	67	No hit	
31 (2980)	rep 15	3	696	1391	232	repeat element protein 5 [HdIV]	AAR89177.1
	rep 13	2	2300	2979	227	repeat element protein-d2.1 [HfIV]	YP_001031304.1
	Hc31-1	-1	665	934	90	No hit	
32 (2881)	rep 21	2	2105	2827	241	repeat element protein-b14.1 [HfIV]	YP_001031359.1
	Hc32-1	-2	2320	2568	83	No hit	
	Hc32-2	-2	250	498	83	No hit	
	Hc32-3	1	2152	2373	74	No hit	
	Hc32-4	-1	1334	1540	69	No hit	
	Hc32-5	-2	1960	2160	67	No hit	
33 (3547)	vinnexin 1	2	2171	3229	353	innexin Vnx-c16 [HfIV]	YP_001031223.1
	Hc33-1	1	2134	2436	101	No hit	
	Hc33-2	2	461	685	75	No hit	
	Hc33-3	1	1171	1380	70	No hit	
	Hc33-4	-2	64	273	70	No hit	
34 (4547)	rep 19	2	1451	2107	219	repeat element protein 7 [HdIV]	AAR89179.1
	rep 18	1	3448	4083	212	repeat element protein-e2.1 [HfIV]	YP_001031346.1
	Hc34-1	1	1021	1272	84	No hit	
	Hc34-2	-3	235	474	80	No hit	
	Hc34-3	-3	3301	3534	78	No hit	
	Hc34-4	-2	1646	1861	72	No hit	
35 (4289)	rep 2	1	37	897	287	repeat element protein-c7.1 [HfIV]	YP_001031283.1
	Hc35-1	-2	2492	2827	112	No hit	
	Hc35-2	2	1538	1786	83	No hit	
	Hc35-3	1	2275	2505	77	No hit	
	Hc35-4	3	2610	2831	74	No hit	
	Hc35-5	-3	496	714	73	No hit	
	Hc35-6	-3	2782	2988	69	No hit	
36 (2459)	Hc36-1	-2	1874	2149	92	No hit	
	Hc36-2	-2	1385	1642	86	No hit	
	Hc36-3	1	1936	2163	76	No hit	
	Hc36-4	-3	397	612	72	No hit	
	Hc36-5	-1	1446	1646	67	No hit	

Supplementary table 2 (continued)

Segment (size, bp)	DfIV genes	Frame	from	to	Length (a.a)	Description	Accession
37 (3744)	HfIV b7.1	2	1109	1831	241	b7.1 [HfIV]	YP_001031227.1
	vinnexin 4	3	3054	3743, 455	380	innexin Vnx-b7 [HfIV]	YP_001031226.1
	Hc37-1	3	1155	1709	185	No hit	
	Hc37-2	2	3314	3613	100	No hit	
	Hc37-3	-3	119	322	68	No hit	
38 (4125)	Hc38-1	2	548	943	132	No hit	
	Hc38-2	1	3292	3618	109	No hit	
	Hc38-3	1	2152	2448	99	No hit	
	Hc38-4	-1	340	621	94	No hit	
	Hc38-5	-1	874	1131	86	No hit	
	HdIV p12 like	3	660	1017	75	p12 [HdIV]	AAF91314.1
	Hc38-6	-3	1325	1531	69	No hit	
39 (2405)	rep 20	-2	596	1303	236	f3.2 [TrIV]	BAF45626.1
	Hc39-1	-2	1586	1795	70	No hit	
	Hc39-2	1	1567	1767	67	No hit	
40 (3773)	cys-motif 2	-1	402	866	274	CcIV 1.0 protein [CcIV]	BAC55881.2
	Hc40-1	-1	2460	2783	108	No hit	
	Hc40-2	-2	3326	3637	104	No hit	
	Hc40-3	2	3161	3397	79	No hit	
41 (2392)	rep 26	3	1272	2045	258	c7-1.1 [TrIV]	BAF45598.1
	Hc41-1	-3	1662	1901	80	No hit	
	Hc41-2	-2	1	240	80	No hit	
	Hc41-3	-2	2089	2319	77	No hit	
42 (4794)	rep 26	1	2725	3498	258	c7-1.1 [TrIV]	BAF45598.1
	rep 16	3	4020	4778	253	c7-1.1 [TrIV]	BAF45598.1
	rep 17	-1	1051	1656	202	c7-1.1 [TrIV]	BAF45598.1
	Hc42-1	-3	3521	3769	83	No hit	
	Hc42-2	-1	3115	3354	80	No hit	
	Hc42-3	-3	3866	4090	75	No hit	
	Hc42-4	2	962	1183	74	No hit	
43 (3540)	DNA pol 3 like	-1	1963	2787	275	DNA polymerase III subunits [*Variovorax paradoxus* S110]	YP_002944223.1
	HfIV e1.3 like	3	2424	2789	122	e1.3 [HfIV]	YP_001031364.1
	H43-1	-2	3063	3344	94	No hit	
	H43-2	-3	89	355	89	No hit	
	H43-3	2	3305	3539	234	No hit	
	H43-4	-2	423	641	73	No hit	
	H43-5	1	118	330	71	No hit	
44 (4158)	vinnexin 6	2	545	1618	358	innexin Vnx-c16 [HfIV]	YP_001031223.1
	vankyrin 5	1	2434	2949	172	vankyrin 2 [HdIV]	AFH35118.1
	Hc45-1	1	1255	1482	76	No hit	
	Hc45-2	-1	2674	2898	75	No hit	
	Hc45-3	3	345	557	71	No hit	

Supplementary table 2 (continued)

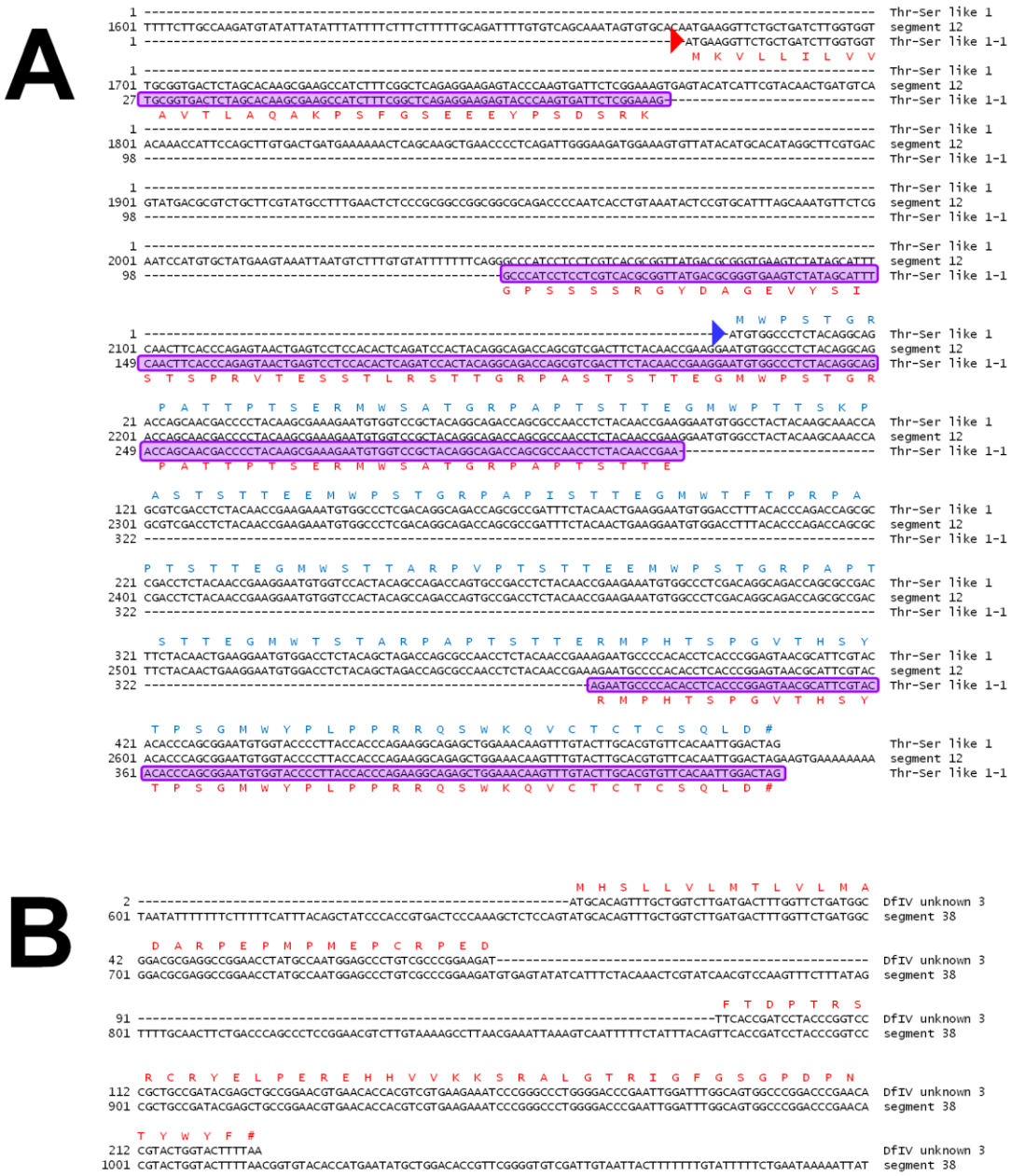
Segment (size, bp)	DfIV genes	Frame	from	to	Length (a.a)	Description	Accession
45 (3243)	rep 12	3	2805	3242, 371	270	c7-1.1 [TrIV]	BAF45598.1
	Hc46-1	2	425	697	91	No hit	
	Hc46-2	-2	1833	2045	71	No hit	
	Hc46-3	3	2589	2795	69	No hit	
46 (2036)	HfIv c17.1 like	-1	921	1988	356	c17.1 [HfIV]	YP_001031291.1
	unknown 10	1	835	1302	156	No hit	
	Hc47-1	2	2	256	85	No hit	
47 (3337)	rep1	2	1028	1705	226	c7-1.1 [TrIV]	BAF45598.1
	rep37	1	2788	3210	141	f3.2 [TrIV]	BAF45626.1
	HfIV d6.1 like	-1	1298	1558	87	d6.1 [HfIV]	YP_001031321.1
	Hc48-1	-2	1207	1416	70	No hit	
	Hc48-2	1	2527	2733	69	No hit	
	Hc48-3	2	1889	2089	67	No hit	
48 (4504)	vinnexin 6, c49	1	2608	3681	358	innexin Vnx-c16 [HfIV]	YP_001031223.1
	Hc49-1	-2	376	1101	242	No hit	
	vankyrin 2	1	496	1014	173	vankyrin 1 [HdIV]	AFH35112.1
	Hc49-2	3	3318	3545	76	No hit	
	Hc49-3	2	2408	2620	71	No hit	
49 (4691)	rep 5	-2	2138	2920	261	f3.2 [TrIV]	BAF45626.1
	rep 11, c50	-1	192	899	236	rep c18.1 [HfIV]	YP_001031294.1
	rep 4	-2	3578	4108	177	f3.2 [TrIV]	BAF45626.1
	HfIV c18.1 like	1	4432	4683	84	c18.1 [HfIV]	YP_001031295.1
	Hc50-1	-2	257	478	74	No hit	
	Hc50-2	-1	954	1160	69	No hit	
50 (1919)	Hc51-1	3	162	392	77	No hit	
51 (2613)	polar residue rich 2	2	1619	1996	126	polar residue-rich protein-b8 [HfIV]	YP_001031235.1
	Hc52-1	-1	328	597	90	No hit	
	Hc52-2	3	1911	2120	70	No hit	
52 (4510)	vinnexin 2	-1	950	2086	379	innexin Vnx-c16 [HfIV]	YP_001031223.1
	Hc53-1	3	2184	2504	107	No hit	
	Hc53-2	1	3874	4131	86	No hit	
	Hc53-3	2	3389	3643	85	No hit	
	Hc53-4	1	2770	2982	71	No hit	
	Hc53-5	-2	1159	1368	70	No hit	
	Hc53-6	-3	3375	3581	69	No hit	
	Hc53-7	3	3870	4070	67	No hit	
	Hc53-8	2	1436	1636	67	No hit	
53 (3320)	rep 36	1	442	909	156	f3.3 [TrIV]	BAF45627.1
	Hc54-1	-2	2468	2719	84	No hit	
	Hc54-2	-1	2865	3113	83	No hit	
	Hc54-3	-1	1131	1379	83	No hit	
	Hc54-4	2	1817	2041	75	No hit	
	Hc54-5	-1	657	881	75	No hit	
	Hc54-6	2	896	1117	74	No hit	
	Hc54-7	3	489	710	74	No hit	
	Hc54-8	-1	297	497	67	No hit	

Supplementary table 2 (continued)

Segment (size, bp)	DfIV genes	Frame	from	to	Length (a.a)	Description	Accession
54 (1749)	HdIV unknown protein 3 like	1	1078	1731	218	unknown [HdIV]	AAO33351.1
55 (3612)	HfIV c6.2 like	-2	2517	2870	118	c6.2 [HfIV]	YP_001031281.1
	Ser B like protein	1	2113	2403	97	phosphoserine phosphatase serB2 [*Mycobacterium tuberculosis*]	WP_003914114.1
	HfIV c6.3 like	-3	104	373	90	c6.3 [HfIV]	YP_001031282.1
	Hc56-1	2	1547	1807	87	No hit	
	Hc56-2	2	1037	1285	83	No hit	
	Hc56-3	-1	3379	3611	78	No hit	
	Hc56-4	-2	1668	1889	74	No hit	
56 (2300)	vinnexin 6	1	988	2061	358	innexin Vnx-c16 [HfIV]	YP_001031223.1
	Hc58-1	3	1698	1925	76	No hit	
	Hc58-2	1	160	387	76	No hit	
	Hc58-3	2	788	1000	71	No hit	
	Hc58-4	-2	113	325	71	No hit	
57 (1511)	Hc59-1	-3	661	891	77	No hit	
	Hc59-2	-1	684	911	76	No hit	
	Hc59-3	3	105	329	75	No hit	
	Hc59-4	-2	443	664	74	No hit	
	Hc59-5	1	1	210	70	No hit	
58 (3539)	Hc60-1	2	620	916	99	No hit	
	Hc60-2	3	588	884	99	No hit	
	HfIV b8.1 like	-2	1640	1900	87	b8.1 [HfIV]	YP_001031236.1
	RnfC like	-1	2535	2789	85	electron transport complex protein RnfC [Klebsiella sp.]	ZP_06548630.1
	Hc60-3	-3	508	741	78	No hit	
59 (2573)	amidase like	-2	1517	2314	266	N-acetylmuramoyl-L-alanine amidase [*Staphylococcus aureus*]	WP_001805448.1
	Hc61-1	-1	975	1235	87	No hit	
	Hc61-2	1	2320	2535	72	No hit	
	Hc61-3	-1	696	896	67	No hit	
60 (1714)	GfIV-c7-ORF2 like	-2	406	1110	235	GfV-C7-ORF2 [GfIV]	YP_001029409.1
	vankyrin 2	1	505	1023	173	vankyrin 1 [HdIV]	AFH35112.1
61 (3745)	vinnexin 4	1	2299	3438	380	innexin Vnx-b7 [HfIV]	YP_001031226.1
	HfIv b7.1 like	2	389	1069	227	b7.1 [HfIV]	YP_001031227.1
	Hc63-1	3	2553	2837	95	No hit	
	Hc63-2	1	343	591	83	No hit	
	Hc63-3	3	1539	1781	81	No hit	
	Hc63-4	-1	2528	2740	71	No hit	
	Hc63-5	-3	3102	3305	68	No hit	
62 (1880)	rep 38	3	1368	1868	167	repeat element protein-d2.1 [HfIV]	YP_001031304.1
	rep 39	2	1193	1432	80	repeat element protein 6 [HdIV]	AAR89178.1
63 (1893)	Recombination inhibitor protein like	-2	1119	1493	125	recombination and DNA strand exchange inhibitor protein [Cyanothece sp. ATCC 51142]	YP_001803304.1
	Hc65-1	-3	1349	1642	98	No hit	

Supplementary table 2 (continued)

Segment (size, bp)	DfIV genes	Frame	from	to	Length (a.a)	Description	Accession
64 (2210)	HfIV c17.1 like	-2	287	850	188	c17.1 [HfIV]	YP_001031291.1
	Hc66-1	3	201	668	156	No hit	
	Hc66-2	-2	1307	1591	95	No hit	
	Hc66-3	-1	1281	1535	85	No hit	
	Hc66-4	-3	646	846	67	No hit	
65 (1426)	cys-motif 6	3	513	1379	289	cysteine motif gene-d9.1 [HfIV]	YP_001031331.1
	Hc67-1	-2	1162	1404	81	No hit	



Supplementary fig. 1. Alternative splicing observed in DfIV genome segment 12 (A) and 38 (B) which encoded thr-Ser like protein and HdIV p12 like protein gene, respectively. Red or blue letter means their deduced amino acid sequence and triangle indicated predicted translation starting point.

Abstract in Korean

감자뿔나방살이자루맵시벌 이크노바이러스의 동정 및 특성구명과 피기생기주내 바이러스 유전체 발현의 가소성

김 주 일

초 록

포식기생은 곤충의 여섯 목(order)에서 발견되며 그 중 벌목 특히 맵시벌상과가 가장 많은 수를 차지한다. 맵시벌상과는 4개과, 6만종 이상을 포함하는 벌목 중 가장 큰 상과이다. 이렇게 종이 다양 할 수 있는 이유는 바로 폴리드나바이러스와 같은 공생 요인이 있기때문이다. 폴리드나바이러스는 Polydnaviridae에 속하며 일부 맵시벌상과내 기생봉에 따라 브라코바이러스 (고치벌과) 와 이크노바이러스 (맵시벌과)로 나뉘게 된다. 본 연구에서는 감자뿔나방살이자루맵시벌 이크노바이러스 (*Diadegma fenestrale* ichnovirus; DfIV) 라고 명명한 새로운 폴리드나바이러스를 감자뿔나방살이자루맵시벌 암컷 난소, 특히 난소받침에서 발견하였다. DfIV는 이중막 구조의 전형적인 이크노바이러스 형태를 보였으며, 조각형 유전체를 갖는 폴리드나바이러스의 특성을 가지고 있었다. 전체 65개의 분리된 유전체 고리를 확인하였으며 247,191bp 전체 염기서열을 읽고 분석하였다. 65개의 유전체 고리의 상대적인 양은 다양했으며 그중 62개가 HfIV와 유사도가 높았고, 평균 GC함량은 43.3% 였다. 전체 99개의 해독틀을 다음과 같이 예측하였다. 40개의 rep, 12개의 cys-motif, 8개의 vankyrin, 6개의 vinnexin, 2개의 polar-residue rich, 1개의 N유전자 그리고 위의 유전자 집단에 포합되지 않는 30개의 유전자. 감자뿔나방살이자루맵시벌은 야외 포장은 물론 실험실에서도 감자뿔나방과 배추좀나방을 기생하는데, 산란수와 생존률을 기준으로하였을 때 기주로서 배추좀나방에 비해서 감자뿔나방을 더 선호하는 것으로 나타났다. 더구나

DfIV는 기생 후 배추좀나방보다 감자뿔나방에서 유전자의 발현이 높은데, 특히 기생 초기에 매우 높았다. 또한 많은 수의 DfIV 유전자가 감자뿔나방에서 주로 발현되었으며, 이러한 유전자들은 두 나비목 기주에서 서로 다른 발현 양상을 보였다. 이 DfIV 유전체 발현의 가소성은 나비목 기주의 종과 기생 후 시간 경과에 따라 나타났다. 또한 이러한 DfIV 유전체 가소성은 그들의 기생봉의 생존률을 높였다. 이것은 PDV와 기생봉간의 공생과 공진화의 증거이며, 새롭게 발견된 DfIV 유전자들은 다양한 연구 분야에 활용이 가능 할 것이다.

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이 논문은 사랑하는 아내와 함께 썼다고해도 과언이 아닐만큼 아내의 도움 없이는 결코 이 논문이 빛을 보지 못했을 것입니다. 7년전 아내를 만나 박사 과정 중에 결혼하고 축복으로 찾아온 두 아이들, 하늘이와 온누리. 논문 준비로 몇일씩 가끔은 열흘 넘게 집에도 못 들어가는 상황에서도 오히려 건강 꼭 챙기라고, 힘내라고 용기를 주었던 아내의 사랑은 평생 잊지 못할 것입니다. 이제는 유리라는 이름보다 하늘이 엄마가 익숙해진 내 사랑. 내가 지금까지 제일 잘 한 일은 당신을 만나 사랑한 일일꺼야. 우리 네 식구 꼭 행복하게 살자. 사랑해요.

아침에 출근할 때 "운전조심해"하며 아쉬운 손을 흔들어주던 하늘이. 이제 겨우 한달을 넘긴 온누리. 아빠가 많은 시간 함께 보내주지 못해 미안해. 더 좋은 아빠가 되도록 노력할께. 너희들이 주는 크고 순수한 사랑이 얼마나 아빠에게는 힘이되는지 모른단다. 하늘아, 누리야 사랑한다.

두 아들의 아빠가되어 보니, 부모님의 사랑에 대해 더 많이 생각하게 되는 것 같습니다. 자식들 공부에는 누구보다 더 열심이셨던 부모님 덕분에 이렇게 또 한 걸음 나아갈 수 있었습니다. 아버지의 갑작스런 폐암 발병으로 육체적으로도 정신적으로도 힘드신 순간에도 늘 자식 걱정이 먼저이셨던 부모님. 사랑합니다. 그리고 존경합니다. 꼭 오래오래 건강하게 살아주세요. 아직 부모님께 배울 것도 그리고 제가 해드리고 싶은 것도 너무나 많답니다.

결혼과 함께 뵙게된 또 한 부모님. 바로 장인어른과 장모님입니다. 늘 인자하시며, 사회생활 힘들지 하시며 힘들 때 물심양면으로 도와주셨던 장님어른. 아내 산후조리는 물론 아이들 돌봐주심까지 자식 뒷바라지에 늘 헌신적이신 장모님. 두 분 모두 존경하고 사랑합니다. 꼭 건강하게 오래오래 살아주세요.

하늘나라에서 저를 지켜봐주실 할아버지와 병환으로 누워계신 할머니. 개구장이였던 손자가 벌써 이렇게 컸네요. 멀리서도 늘한결 같은 마음으로 응원해주던 누나네와 동생네 식구들. 자주 만나지는 못하지만 늘 고마워요. 한 동네에서 한마음으로 저를 응원해주신 작은할아버지, 큰아저씨, 작은아버지, 큰외삼촌 그리고 멀리서도 저를 응원해주신 친척들과 처가 친척들께도 깊은 감사를 드립니다.

곤충 분자생물학이라는 분야를 처음 접하던 석사때부터 지금까지 한결 같은 모습으로 저를 이끌어주시는 이시혁 교수님. 교수님의 지도가 없었으면 아마 지금의 저도 없었겠죠. 혼자 자취하던 석사때는 집에 식사를 초대해 주실 만큼 잘 챙겨주셨고, 파트로 다니던 박사 과정 때는 바쁜 시간 쪼개가며 실험과 논문을 꼼꼼하게 봐주시던 교수님. 정말 감사합니다. 앞으로도 더 노력하는 마음으로 교수님의 발자취를 따라가고자 합니다. 이번 논문의 위원장을 맡아주셨던 제연호 교수님. 늘 큰 형님처럼 잘 챙겨주셔서 감사합니다. 세계적인 PDV의 대가로서 처음 이 감자뿔나방살이자루맵시벌 난소 해부 한 사진을 보고 한 눈에 그 가치를 알아봐주시고 연구를 독려해 주셨던 안동대 김용균 교수님. 정말 교수님 덕분에 이 논문이 나오게 되었습니다. TEM 사진은 물론 연구의 방향과 앞으로의 방향까지도 늘 진실된 마음으로 저를 이끌어 주셔서 감사합니다. 심사의원으로 날카로운 조언과 따뜻한 격려를 아끼지 않아주셨던 권형욱 교수님과 이광범 교수님게도 감사의 인사를 올립니다. 10년 가까이 곤충학과 교수님으로서뿐만 아니라 선배 연구자로서도 늘 좋은 모습을 보여주셨던 안용준 교수님과 이준호 교수님 그리고 이승환 교수님 감사합니다.

처음 곤충학을 접하던 학부때 저를 이끌어주셨던 김길하 교수님. 교수님은 저에게는 정말 아버지이기도 큰 형님이기도 합니다. 교수님과 사모님께 깊은 감사의 인사를 올립니다. 해부라는 새로운 분야를 접하게 해주신 한림대 고영호 교수님. 때론 형님처럼 저를 이끌어주시고 다독여주셔서 감사합니다.

저는 지금 농촌진흥청 국립식량과학원 고령지농업연구센터에서 일하고 있습니다. 이곳에 처음 발령받아오면서 좁기만 했던 저의 시야가 이렇게 조금아니마 넓어지게 되었습니다. 농업, 농촌에 대해 더 많이 이해하고 벌레와 그 공생생물들에 대해서도 관심을 가지게 되었습니다. 좁은가슴잎벌레와 그레가린이 저의 첫 도전이었고, 옆에서 늘 용기와 힘을 주셨던 권민 박사님께서 연구하시던 기생봉은 저의 두번째 도전이었습니다. 그러던 중 운명처럼 감자뿔나방살이자루맵시벌을 만났고 그로 인해 좋은 사람들의 인연도 만났습니다. 저의 영원한 실장님 권민 박사님과 기생봉 분류를 맡아주셨던 영남대 이종욱 교수님과 최진경 박사님 정말 감사합니다. 곤충사육의 마이더스의 손 홍은주 여사님과 오랜시간 동안 저를 밑고 따라준 심재동, 김성희 연구원 모두 감사합니다. 두번의 방학기간 알바와서 논문 실험 많이 도와주었던 장윤기 학생에게도 감사의 인사를 합니다. 저에게는 소중한 직장인 이곳에 참 고마운 분들이 많네요. 언제나 따스한 격려를 보내주신 정진철 소장님을 비롯한 전 직원께 깊은 감사를 드립니다.

이웃사촌으로, 인생의 선배로 따스한 조언으로 용기를 주셨던 최영웅, 최종인 교수님과 사모님들께도 감사를 드립니다.

실험실에서 함께 연구에 매진하는 선후배님들에게도 감사의 인사를 합니다. 실험실 맡형 권덕호 박사님, 산림과학원의 강재순 박사님, 찬식이형, 연세대의 백지형 박사, 실험실장 김영호 박사, 유학 중인 건묵이, 아빠가 된 정훈이, 실험실에서 참 많이 도와주었던 진균이, 지선이, 소영이, 지현이, 덕재, 주현이, 채은, 경재. 모두 고마워. 처음 PDV라는 주제로 연구를 시작 했을때부터 지금까지도 많은 도움을 주고 있는 안동대 김용균 교수님 실험실의 많은 동료 연구자들과 제가 처음 곤충을 주제로 연구를 시작 할 수 있었던 충북대 김길하 교수님 실험실의 많은 선후배님들께도 감사의 인사를 드립니다.

이 논문뿐 만 아니라 저에게는 작은 전환점이었던 2012 ICE에서 심포지엄 연사의 기회를 주었던 Ian Denholm, Ralf Nauen, 기생봉 분자 분류 등에 조언을 주었던 Balmer Oliver에게도 감사를 드립니다. 그외에도 응용곤충학회 심포지엄 연사의 기회를 주셨던 경상대 이대원 교수님, 유전자 진화 연구에 많은 도움을 준 분류실 소라, 활란이, 신승관 박사 등 많은 분들께 감사의 인사를 올립니다. 그외에도 정말 많은 분들께서 도움을 주셨습니다. 일일이 말씀드리지 못하더라도 그 고마움. 마음에 꼭 간직하겠습니다.

사랑의 하나님. 이렇게 미약하나마 작은 노력의 결실을 글로 적었습니다. 어려울때나 기쁠때나 늘 함께해주심에 감사드리며, 앞으로도 저에게 열정을 주시어 주님께서 바라시는 바를 이루게 하소서.

멋모르고 벌레가 좋아 시작한지 언 15년. 이제 저의 인생에 한 부분이 되어 저를 꿈꾸게 만들어주는 이 생명체들에게 고맙다는 말을 꼭 하고싶습니다. 그 생명체를 바라보고 있노라면 하나님의 경이로운 솜씨에 놀라곤 합니다. 엄청난 다양성, 유용 유전자들의 보물창고 그리고 진화라는 미지의 세계까지. 저의 능력이 닿는 한 이 생명체에게 끊임없이 물음을 던지고 싶습니다. 그리고 학자로서 부끄럽지 않은 삶을 살아가도록 노력하고자 합니다. 감사합니다.

이천십삼년 칠월의 끝자락에

대관령에서

김주일