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# Comparative analysis of the genome and host range characteristics of two insect iridoviruses: Chilo iridescent virus and a cricket iridovirus isolate

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**The iridovirus isolate termed cricket iridovirus (CrIV) was isolated in 1996 from *Gryllus campestris* L. and *Acheta domesticus* L. (both Orthoptera, Gryllidae). CrIV DNA shows distinct DNA restriction patterns different from those known for *Insect iridescent virus type 6* (IIV-6). This observation led to the assumption that CrIV might be a new species within the family *Iridoviridae*. CrIV can be transmitted perorally to orthopteran species, resulting in specific, fatal diseases. These species include *Gryllus bimaculatus* L. and the African migratory locust *Locusta migratoria migratorioides* (Orthoptera, Acrididae). Analysis of genomic and host range properties of this isolate was carried out in comparison to those known for IIV-6. Host range studies of CrIV and IIV-6 revealed no differences in the peroral susceptibility in all insect species and developmental stages tested to date. Different gene loci of the IIV-6 genome were analyzed, including the major capsid protein (274L), thymidylate synthase (225R), an exonuclease (012L), DNA polymerase (037L), ATPase (075L), DNA ligase (205R) and the open reading frame 339L, which is homologous to the immediate-early protein ICP-46 of frog virus 3. The average identity of the selected viral genes and their gene products was found to be 95·98 and 95·18% at the nucleotide and amino acid level, respectively. These data led to the conclusion that CrIV and IIV-6 are not different species within the *Iridoviridae* family and that CrIV must be considered to be a variant and/or a novel strain of IIV-6.**

## Introduction

Iridoviruses are large, icosahedral, cytoplasmic deoxyriboviruses: a major characteristic of iridoviruses is their large icosahedral capsid and their replication and assembly in the cytoplasm. Iridoviruses have been isolated from insects of the orders Lepidoptera, Diptera, Hemiptera, Hymenoptera and Coleoptera (Tinsley & Kelly, 1985; Kelly, 1985; Williams, 1994, 1996). There have been only a few reports of iridoviruses isolated from orthopteran species, two from mole crickets (*Scapteriscus* species) and the rest from crickets (Boucias *et al.*, 1987; Fowler, 1989; Kleespies *et al.*, 1999; Just & Essbauer, 2001).

Iridoviruses are subdivided into four genera: *Iridovirus*, *Chloriridovirus*, *Lymphocystivirus* and *Ranavirus* (van Regenmortel *et al.*, 2000). The type species for the genus *Iridovirus* is *Insect iridescent virus type 6* (IIV-6 or the recognized synonym Chilo iridescent virus). IIV-6 was isolated from the stem-boring lepidopteran *Chilo suppressalis* (rice-stem borer; Fukaya & Nasu, 1966). Recently, we succeeded in identifying the primary structure and coding strategy of the complete genome of IIV-6 (Jakob *et al.*, 2001).

The cricket iridovirus isolate (CrIV) was isolated in 1996 from *Gryllus campestris* L. and *Acheta domesticus* L. (both Orthoptera, Gryllidae) (Kleespies *et al.*, 1999). It was found that the DNA restriction pattern of CrIV DNA is distinct from that of IIV-6 (Kleespies *et al.*, 1999) but seems to be identical to the restriction patterns of the CrIV isolate described by Just & Essbauer (2001). Host range studies revealed that CrIV can be transmitted perorally to orthopteran species, resulting in characteristic symptoms and fatal disease (Kleespies *et al.*, 1999). The orthopteran species include *Gryllus bimaculatus* L. (Orthoptera, Gryllidae) and the African migratory locust

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*Locusta migratoria migratorioides* (Reiche & Fairmaire) (Orthoptera, Acrididae), which represent important insect pests in some developing countries. Furthermore, CrIV is lethal for first instars of the cockroaches *Blattella germanica* L. and *Blatta orientalis* L. (both Orthoptera, Blattidae) (Kleespies *et al*., 1999).

A common feature of CrIV and IIV-6 is the ability to grow in CF-124 (Lepidoptera, *Choristoneura fumiferana*) cell cultures. Cell lysis, rounding and hypertrophy of the usually bipolar cells were observed within 24 h post-infection.

The molecular biology and genomic features of IIV-6 are well documented (Cerutti *et al*., 1981; Cerutti & Devauchelle, 1980, 1982, 1985, 1990; Delius *et al*., 1984; Schnitzler *et al*., 1987; Soltau *et al*., 1987; Fischer *et al*., 1988a, b, 1990; Sonntag & Darai, 1992, 1995; Handermann *et al*., 1992; Stohwasser *et al*., 1993; Sonntag *et al*., 1994a, b; Bahr *et al*., 1997; Tidona & Darai, 1997, 2000; Tidona *et al*., 1998; Müller *et al*., 1998, 1999; Jakob *et al*., 2001).

The present study is a comparative analysis of the host range and genomic properties of CrIV and IIV-6.

## Methods

■ **Virus and cells.** IIV-6 was propagated in CF-124 cell cultures, as described previously (Fukaya & Nasu, 1966; Delius *et al*., 1984). CrIV DNA was derived from infected *Gryllus bimaculatus*, as described recently (Kleespies *et al*., 1999).

■ **Host range studies.** To analyze host range infection, experiments were performed using a modification of the method described by Kleespies *et al*. (1999). In the present work, host range experiments were conducted separately with the two iridoviruses, CrIV and IIV-6, together with 19 different orthopteran and lepidopteran species. The following species were tested for susceptibility: crickets, *Acheta domesticus* L., *Gryllus campestris* L., *Gryllus bimaculatus* L., *Gryllus assimilis* Fabricius and *Gryllotalpa gryllotalpa* L. (a mole cricket); locusts and grasshoppers: *Locusta migratoria migratorioides* (Reiche & Fairmaire), *Schistocerca gregaria* Forskål and *Tachycines asynamorus* Adel.; cockroaches: *Blattella germanica* L., *Blatta orientalis* L., *Periplaneta americana* L., *Pycnoscelus surinamensis* L. and *Leucophaea maderae* L.; and butterflies: *Spodoptera exigua* Hbn., *Spodoptera littoralis* Boisd., *Heliothis armigera* Hbn., *Agrotis segetum* Schiff., *Galleria mellonella* F. and *Lymantria dispar* L. Peroral transmission studies were carried out with various nymphal/larval instars and/or adults. For this purpose, virus contaminated food-like pieces of carrot (crickets and cockroaches), pieces of apple (*Tachycines asynamorus* and *Grylus assimilis*), wheat germ (locusts), oak leaves (*Lymantria dispar*) or different diets (all other lepidopteran larvae) were offered. The food was contaminated with a virus suspension of $2{\cdot}2 \times 10^{11}$ particles/ml CrIV or IIV-6 and was provided for 1 day. Virus concentration was determined by direct particle count, as described by Geister & Peters (1963). To avoid cannibalism in each test, 20 nymphs, larvae or adults were placed singly in beakers of different size depending on the size of the insect (diameter 6·5 or 7·5 cm, height 4·5 or 9·5 cm). The control group comprised 10 nymphs, larvae or adults and each species was fed with uncontaminated food. All specimens were incubated at a day/night regime of 25/20 °C. Bioassays were repeated two times and mortality was recorded daily up to 50 days post-inoculation. For verifying the causal agent, fat body preparations from dead specimens were investigated by negative-staining electron microscopy. In the same way, surviving insects were diagnosed for the presence or absence of virus infection.

■ **Electron microscopy.** To study and compare fine structures and morphogenesis of the two viruses, series of fat body samples of diseased animals were dissected and fixed in 2·5% glutaraldehyde. Specimens were prepared for thin section transmission electron microscopy (TEM) following published procedures (Gelderblom *et al*., 1987; Yan *et al*., 2000). Briefly, after post-fixation with 1% osmium tetroxide for 2 h, samples were treated with 0·1% tannic acid for 2 h followed by en bloc-staining with 2% uranyl acetate for 2 h and stepwise dehydration in increasing concentrations of ethanol. Samples were embedded in Epon. Ultrathin sections were obtained using a Leica Ultracut S microtome, post-stained with lead citrate and evaluated under a ZEISS EM 10 A TEM.

Measurements of virus particles were performed on negatives taken at 40000 magnification using a 10× magnifying glass. The 'average' diameters of IIV-6 and CrIV were determined by measuring 100 particles in two directions, i.e. the long and short axes.

■ **DNA isolation.** DNA isolation, incubation and electrophoresis were carried out as described previously (Schnitzler *et al*., 1987; Soltau *et al*., 1987; Fischer *et al*., 1988a, b; Kleespies *et al*., 1999).

■ **PCR.** Oligonucleotide primers were synthesized with an Oligo 1000 M DNA Synthesizer (Beckman). PCR was performed using 0·5 fmol of the individual template DNA in 100 µl volumes containing 80 mM Tris–HCl pH 8·9, 20 mM $(NH_4)_2SO_4$, 5 mM $MgCl_2$, 12·5 nmol of each dNTP, 5 pmol of each primer and 2·5 units *Taq* DNA polymerase (Applied Biosystems). A total of 35 cycles was run on an automated temperature cycling reactor (GeneE) under cycling conditions of 96 °C for 30 s, 55 °C for 1 min and 72 °C for 2 min per cycle.

■ **DNA sequencing.** DNA templates and PCR products were prepared as described previously (Tidona & Darai, 1997). PCR products of the viral genomic DNA were automatically sequenced (Smith *et al*., 1986) with a 373A 'Extended' DNA sequencer using the DyeDeoxy Terminator *Taq* cycle sequencing technique (Applied Biosystems). Sequencing reactions were performed as described previously (Tidona & Darai, 1997). The nucleotide sequences obtained from individual sequencing reactions were assembled using the Sequence Navigator software, version 1·0 (Applied Biosystems).

■ **Computer-assisted analysis.** Nucleotide and amino acid sequences were compiled and analyzed using the OMIGA v2 (Oxford Molecular) and Vector NTI Suite 6 (InforMax) and PC/GENE program release 6·85 (Intelligenetics). Protein alignments were generated using the CLUSTAL program (Higgins & Sharp, 1988).

## Results

### Host range studies

Comparative studies on the host range of CrIV and IIV-6 revealed that, as far as tested, there were no differences between the two virus isolates in the peroral susceptibility of all insect species and developmental stages (Table 1). First and third instars of three cricket species tested, *Acheta domesticus*, *Gryllus campestris* and *Gryllus bimaculatus*, were highly susceptible as well as the first instar of a desert cricket, *Gryllus assimilis*. Adults of the mole cricket, *Gryllotalpa gryllotalpa*, were not susceptible to CrIV, while this species was not tested with IIV-6. Young nymphs of the two most economically important locusts, the African migratory locust *Locusta migratoria migratorioides* and the desert locust *Schistocerca gregaria*,

**Table 1.** Host range of CrIV and IIV-6 and susceptibility to individual species and developmental stages of host insects

This table was completed using data from Kleespies *et al.* (1999). L, larvae; NT, not tested; +, susceptible; –, not susceptible.

| Species | Host state | Susceptibility | |
|---|---|---|---|
| | | CrIV | IIV-6 |
| **Orthoptera** | | | |
| Crickets: | | | |
| *Acheta domesticus* L. (Gryllidae) | $L_1$ | + | + |
| | $L_3$ | + | + |
| *Gryllus campestris* L. (Gryllidae) | $L_1$ | + | + |
| | $L_3$ | + | + |
| *Gryllus bimaculatus* L. (Gryllidae) | $L_1$ | + | + |
| | $L_3$ | + | + |
| *Gryllus assimilis* Fabricius (Gryllidae) | $L_1$ | + | NT |
| Mole cricket | | | |
| *Gryllotalpa gryllotalpa* L. (Gryllotalpidae) | Adult | – | NT |
| Locusts and grasshoppers: | | | |
| *Locusta migratoria migratorioides* (Reiche & Fairmaire) (Acrididae) | $L_1$ | + | + |
| | $L_3$ | + | + |
| *Schistocerca gregaria* Forskål (Acrididae) | $L_2$ | + | + |
| *Tachycines asynamorus* Adel. (Rhaphidophoridae) | $L_2$ | – | NT |
| Cockroaches: | | | |
| *Blattella germanica* L. (Blattidae) | $L_1$ | + | + |
| | $L_3$ | – | – |
| *Blatta orientalis* L. (Blattidae) | $L_1$ | + | + |
| | Adult | – | – |
| *Periplaneta americana* L. (Blattidae) | $L_1$ | + | NT |
| *Pycnoscelus surinamensis* L.(Pycnoscelidae) | $L_3$ | – | – |
| *Leucophaea maderae* L. (Oxyphaloidae) | $L_3$ | – | – |
| **Lepidoptera** | | | |
| *Galleria mellonella* F. (Pyralidae) | $L_3$ | + | + |
| *Spodoptera exigua* Hbn. (Noctuidae) | $L_1$ | – | – |
| *Spodoptera littoralis* Boisd. (Noctuidae) | $L_1$ | – | – |
| *Heliothis armigera* Hbn. (Noctuidae) | $L_1$ | – | – |
| *Agrotis segetum* Schiff. (Noctuidae) | $L_1$ | – | – |
| *Lymantria dispar* L. (Lymantriidae) | $L_3$ | – | NT |

($L_1$–$L_3$), were also heavily infected by the two virus isolates. In contrast, the grasshopper *Tachycines asynamorus*, which causes severe damage, especially in glasshouse cultures, proved to be resistant against CrIV. A further interesting perspective was the susceptibility of first instars of two pest insect species, the cockroaches *Blattella germanica* and *Blatta orientalis*, to CrIV and IIV-6. First instars of the American cockroach, *Periplaneta americana*, were infected with CrIV and showed susceptibility. In this case, the IIV-6 isolate was not tested. Only one of the six lepidopteran species tested, the greater wax moth *Galleria mellonella*, was susceptible to both virus isolates, while no virus infection could be observed in all species of the insect order Noctuidae (*Spodoptera exigua*, *Spodoptera littoralis* and *Agrotis segetum*) and in the gypsy moth *Lymantria dispar*.

## Electron microscopy

Ultrathin section TEM in the cytoplasm of grossly enlarged fat cells revealed numerous IIV-6 and CrIV particles, sometimes in dense, paracrystalline arrays. Depending on the section plane, an angular, isometric outline pointing to icosahedral symmetry of the prominent virus capsids became apparent. The capsids bear a dense fringe of surface protrusions on the outside and are completely filled with a slightly electron-opaque matter that, in case of CrIV, started to condense into an electron-dense inner body. Principally, as suggested also by the size measurements of the capsids, there are no major differences between IIV-6 and CrIV.

To determine the dimensions of CrIV and IIV-6 in ultrathin sections, the diameters of the capsids were measured. CrIV showed a long (apex-to-apex) axis of $145 \pm 6$ nm and a short axis of $131 \pm 5$ nm, while IIV-6 measured $142{\cdot}5 \pm 6$ nm and $126{\cdot}5 \pm 5$ nm, respectively. The subtle differences observed here are insignificant and point to identical sizes. When compared in detail (Fig. 1), CrIV and IIV-6 did not show any apparent morphological differences.

## Analysis of genomic properties of CrIV

In order to determine the relatedness of CrIV to the iridoviruses, the DNA nucleotide sequence of the viral gene locus of the major capsid protein (MCP) was determined by PCR. Seven DNA oligonucleotide primers corresponding to the known genomic region of the MCP of IIV-6, which is conserved among the iridoviruses (Tidona *et al.*, 1998), were synthesized. The properties of the oligonucleotide primers used in this study are given in Table 2. Using this strategy, 333 codons of the CrIV MCP gene locus (999 nt) were determined. This region of the CrIV genome corresponds to nt 83–1071 within the DNA nucleotide sequence of the gene locus of IIV-6 MCP. Alignment of the DNA nucleotide sequence of this particular region of CrIV and IIV-6 showed 97·7% identity (Fig. 2A). The number of DNA nucleotide exchanges detected was found to be 23. This corresponds to 7 out of 333 amino acid changes (2·1%) within the deduced amino acid sequence of CrIV MCP (Fig. 2B). As shown in Fig. 2(B) and Table 3, four amino acid exchanges are well-conserved substitutes. The region from nt 372 to 716 (Fig. 2A) of CrIV showed 100% identity at the nucleotide level to the CrIV isolate described by Just & Essbauer (2001). Due to the results obtained from the analysis of the MCP gene, it was necessary to verify these findings by analysing further viral genes of IIV-6 and CrIV. Different distinct gene loci of the IIV-6 genome, which was elucidated completely by Jakob *et al.* (2001), including thymidylate synthase (225R), an exonuclease (012L), DNA

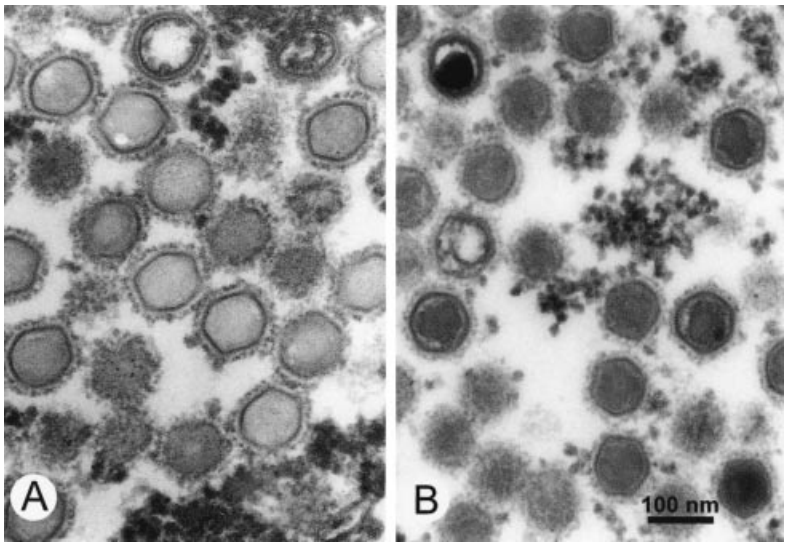


Fig. 1. Electron micrographs of ultrathin sections of IIV-6 (A) and CrIV (B) particles. Virus capsids, often angular in outline, contain electron-opaque masses, which, in the case of CrIV, are partly condensed. Capsids are surrounded by a dense fringe of surface protrusions. Bar, 100 nm.

**Table 2.** Properties of DNA oligonucleotide primers used for identification of the homologous genes of CrIV

| Genomic region of IIV-6 (AF303741) | | Size | | | |
|---|---|---|---|---|---|
| ORF | Description | aa | nt | Primer label | Sequence 5′→3′ |
| 274L 128758–130158 | IIV-1 MCP (P18162) | 467 | 1401 | MCP-F1 | GGTTTCATCGATATCGCCAC |
| | | | | MCP-F2 | GAAATTGAAAAATATATGTA |
| | | | | MCP-F3 | GGGCCGGAGATTATTTGTT |
| | | | | MCP-R1 | TTCTCTGATTAAGTTATGCAT |
| | | | | MCP-R2 | AGCAGAAACATTTCCAATCAT |
| | | | | MCP-R3 | GACAATAGCATAGTTACCCCA |
| | | | | MCP-R4 | GAAAAGTAATCACTGCCCAT |
| 225R 104095–104979 | Thymidylate synthase of herpesvirus ateles (P12462) | 295 | 885 | PIV-TYSY-C1 | TGACGATAGAACAGGAATTCCAAC |
| | | | | PIV-TYSY-C2 | GTTTTCAGTGGAGACATTATGGTG |
| | | | | PIV-TYSY-C3R | TACAATGACAAGGAGGAAGTGCC |
| | | | | PIV-TYSY-C4R | CGCCATTTCCATTTCTAAAGCTGG |
| 012L 2901–4772 | Exonuclease II of *Schizosaccharomyces pombe* (P40383) | 624 | 1872 | 012L-R1 | ATATGACCATTCCCAATCTGGAAC |
| | | | | 012L-F1 | GGTTGATGATACAGCAGAATGGTC |
| 037L 12470–16288 | DNA polymerase of lymphocystis disease virus (L53484) | 1273 | 3819 | 037L-R1 | TGATCCTAAAAAATGTTTCGCCAC |
| | | | | 037L-F1 | GAAGGAAGAGGAGCGTTAGCT |
| 075L 31678–32451 | Putative protein 054R (ATPase) of lymphocystis disease virus (L63545) | 258 | 774 | 075L-R1 | TCATGGCATCCAAATTTCATATCTC |
| | | | | 075L-F1 | GTCAGACACACAAGAATATAGCAT |
| 205R 89498–91342 | DNA ligase of *Borrelia burgdorferi* (051502) | 615 | 1845 | ′205R-R1 | CTACAATTCAGCAATCTCCTTTCC |
| | | | | 205R-F1 | TATTTCATCAAGGACAAATGTTTACC |
| 393L 174414–175775 | Immediate-early protein ICP-46 of frog virus 3 (P14358) | 454 | 1362 | 393L-R1 | TTGAACCATTCATGTAATTCAAGCAA |
| | | | | 393L-F1 | ATAAGTGGATTATTACAACTCATCGA |

polymerase (037L), ATPase (075L), DNA ligase (205R) and the open reading frame (ORF) 339L, showing significant identity to the immediate-early protein ICP-46 of frog virus 3, were selected for a comparative analysis of these viral genomic regions. The positions of the individual genes on the IIV-6 genome are shown in Fig. 3. The DNA nucleotide sequence of these particular regions of the CrIV genome was determined using a collection of DNA oligonucleotide primers synthesized to correspond to the individual regions of IIV-6 genome (Table 2). The results of these analyses are summarized in Table 3. The

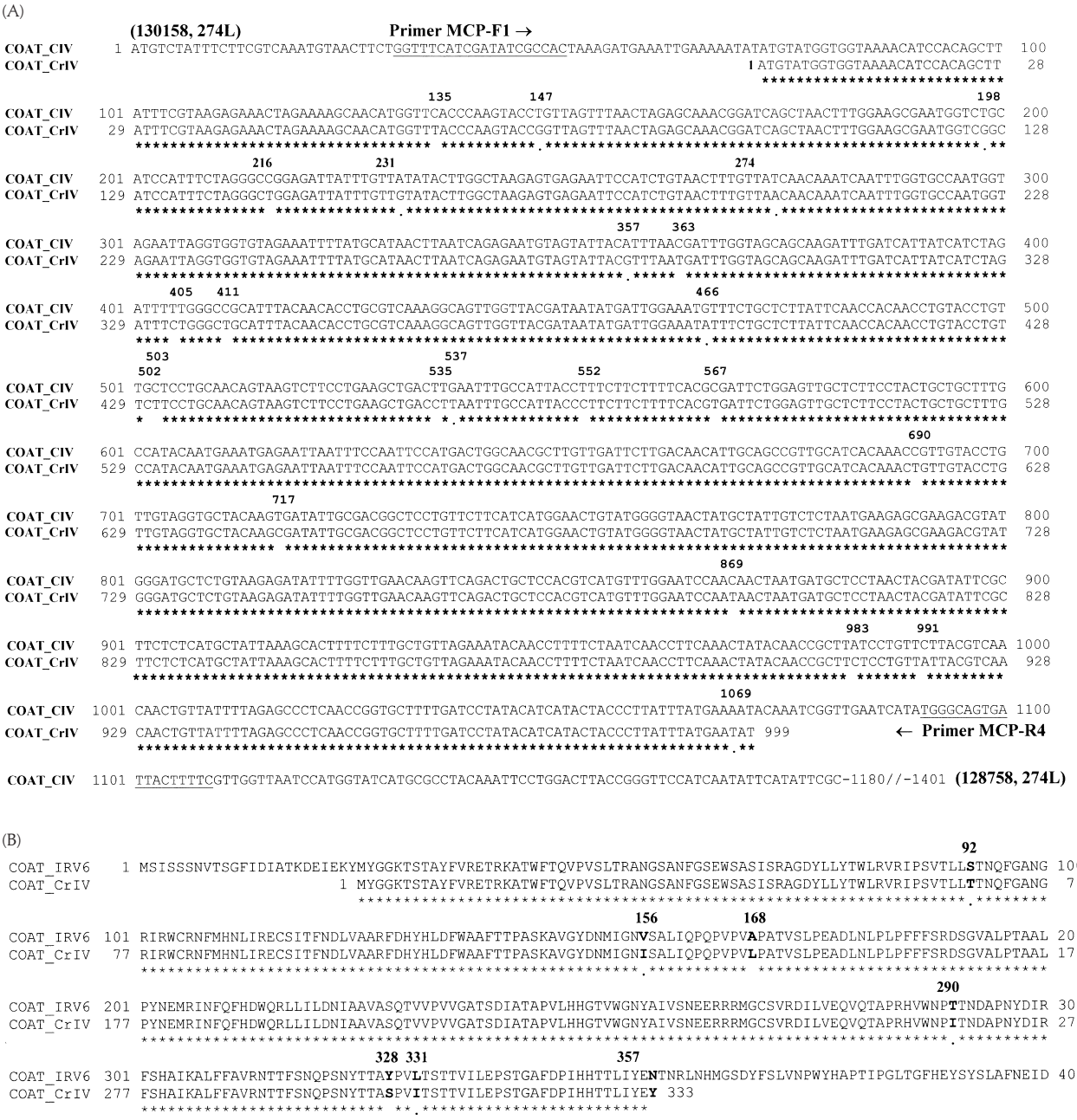


Fig. 2. (A) Alignment of the overlapping DNA nucleotide sequences of 999 nucleotides of the MCP gene of IIV-6 (274L) (COAT-CIV) and CrIV (COAT-CrIV) between nt 83 and 1071 within the DNA nucleotide sequence of the MCP gene of IIV-6. Asterisks indicate identical nucleotides and dots indicate synonymous nucleotide exchanges. The positions of the 23 (2·3%) nucleotide changes are indicated above the nucleotides. (B) Alignment of the overlapping amino acid sequences of 333 amino acid residues of the MCP gene of IIV-6 (274L) (COAT-CIV) and CrIV (COAT-CrIV) between aa 24 and 357 within the amino acid sequence of the MCP gene of IIV-6. Bold letters indicate amino acid exchanges. Asterisks indicate identical amino acids and dots indicate well conserved amino acids. The positions of the seven (2·1%) amino acid changes are indicated above the amino acids.

average identity of the DNA nucleotide sequence of the analyzed genomic region of IIV-6 and CrIV and the average identity of the amino acid residues of the corresponding gene products of these particular loci were found to be 95·98 and 95·18%, respectively; i.e. these results confirm the high identity obtained in the MCP gene analysis described above.

## Discussion

Comparative studies of the infectivity of the novel CrIV isolate and IIV-6 were carried out with different insect species at different developmental stages. In order to determine the genetic relatedness of these viruses, a comparative analysis of

**Table 3.** Nucleotide and amino acid exchanges detected between different ORFs and gene products of the homologous genes of IIV-6 and CrIV

| Genomic region of IIV-6 (AF303741) | | CrIV homologues | | | Number and percentage of nucleotide and amino acid exchanges | | | |
|---|---|---|---|---|---|---|---|---|
| | | Size of PCR product (nt) | Identity (%) | | Total number (%) | Number of synonymous exchanges (%) | Total number | Number of conserved exchanges (%) |
| IIV-6 ORFs | Description | | nt | aa | | | | |
| 274L 128758–130158 | MCP of IIV-1 (P18162) | 999 | 97·7 | 97·9 | 23 (2·3) | 7 (30·43) | 7 (2·1) | 4 (57·14) |
| 225R 104095–104979 | Thymidylate synthase of herpesvirus ateles (P12462) | 732 | 97·3 | 95·2 | 20 (2·7) | 15 (75) | 13 (4·8) | 11 (84·6) |
| 012L 2901–4772 | Exonuclease II of *Schizosaccharomyces pombe* (P40383) | 702 | 94·3 | 95·73 | 40 (5·7) | 13 (32·5) | 10 (4·27) | 3 (30) |
| 037L 12470–16288 | DNA polymerase of lymphocystis disease virus (L63545) | 601 | 93·2 | 89·5 | 41 (6·8) | 18 (43·9) | 21 (10·5) | 16/6·2) |
| 075L 31678–32451 | Putative protein 054R (ATPase) of lymphocystis disease virus (L63545) | 679 | 96·5 | 96·9 | 24 (3·5) | 19 (79·2) | 7 (3·1) | 6 (85·7) |
| 205R 89498–91342 | DNA ligase of *Borrelia burgdorferi* (O51502) | 682 | 94·7 | 95·15 | 36 (5·3) | 12 (33·3) | 11 (4·85) | 5 (45·45) |
| 393L 174414–175775 | Immediate-early protein ICP-46 of frog virus 3 (P14358) | 661 | 98·2 | 95·9 | 12 (1·8) | 5 (41·7) | 7 (3·2) | 7 (100) |

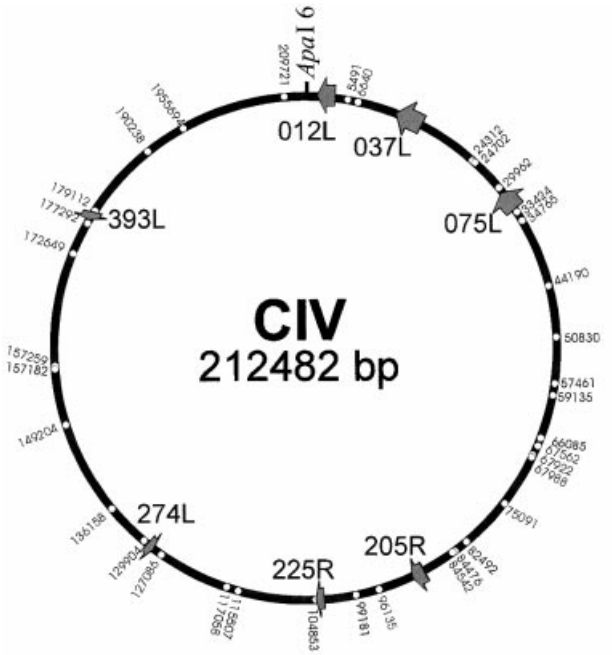


Fig. 3. The position, size and direction of the selected ORFs used in this study are indicated in the physical map of IIV-6. These ORFs include an exonuclease (012L), DNA polymerase (037L), ATPase (075L), DNA ligase (205R), thymidylate synthase (225R), MCP (274L) and ORF 339L, which shows identity to the immediate-early protein ICP-45 of frog virus 3. The positions of the *Eco*RI restriction sites are indicated with white dots.

selected viral putative gene products was performed in which the nucleotide sequence of the different viral genes and the amino acid composition of the corresponding gene products were compared to each other.

Peroral transmission tests were conducted instead of infecting the insects by injection (Ohba, 1975; Anthony & Comps, 1991; Carter, 1973a, b, 1974; Smith, 1976), because this method is irrelevant, both in nature and for potential biological control. It was found that CrIV and IIV-6 have a broad and identical orthopteran host range. The same results were found when *Galleria mellonella*, a member of Lepidoptera, was infected. However, infection tests with several noctuids failed. A common feature of the species tested is the fact that they are known to cause severe damage in different cultures, e.g. sugar cane or cotton. As far as the host range of IIV-6 is concerned, Fukuda (1971) succeeded in perorally infecting 13 species of mosquitoes and Ohba (1975) infected 65 species of Lepidoptera by injection, eight species of Coleoptera and two species (Diptera and Orthoptera) of Hymenoptera.

The average identity of several viral genes and their gene products that where investigated was found to be 95·98 and 95·18% at the nucleotide and amino acid level, respectively. These data and the results obtained from host range studies led to the conclusion that CrIV and IIV-6 are not different species within the *Iridoviridae* family. CrIV must be considered to be a variant and/or a novel strain of IIV-6.

In light of the biological relevance of these viruses for pest control, the results of the present study are of particular importance, as both virus isolates are able to infect different species of Orthoptera, such as the two important pest insects in some developing countries, the African migratory locust *Locusta migratoria migratorioides* and the desert locust *Schistocerca gregaria*.

Although IIV-6 also infects predatory and beneficial insects/invertebrates, including the silkworm *Bombyx mori* (Ohba, 1975), it could provide the basis for, or be a model for, the development of a biological insecticide for pest control. Bioassays with *Locusta migratoria migratorioides* in the laboratory have already delivered promising results (Kleespies *et al*., 1999). Also, the virus susceptibility of first instars to three cockroach species (*Blattella germanica*, *Blatta orientalis* and *Periplaneta americana*) is of practical interest. Therefore, further bioassays are planned with different stages of the virus-susceptible pest insects under special climatic conditions to elucidate the control potential of the two virus isolates.

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