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COMPARATIVE BIOCHEMICAL CHARACTERIZATION OF DIFFERENT WILD ISOLATES OF A NUCLEAR POLYHEDROSIS VIRUS OF *LYMANTRIA DISPAR*

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RESUM

Nou isolats de virus de la polihedrosis nuclear (NPV) de *Lymantria dispar*, procedents de diferents àrees geográfiques d'Europa i Amèrica, han estat caracteritzats bioquimícament i comparats. Els nou isolats no mostraven diferències per la SDS-PAGE en els polipeptids estructurals. Les anàlisi del DNA víric amb els enzims de restricció HindIII, EcoRI i BamHI donen models de fragmentació diferents per als nou isolats. Un d'aquests va ser identificat com una mescla de dues altres variants genotípiques. El fet de presentar mobilitat similar en alguns segments de DNA de cada digestió, suggereix que els nou isoltas pertanyen a soques relacionades. Les homologies que mostren els nou isolats no s'han relacionat amb els seus orígens geogràfics.

ABSTRACT

Nine isolates of a nuclear polyhedrosis virus from *Lymantria dispar* found in different geographical areas in Europe and in North America have been biochemically characterized and compared. The nine isolates showed no differences in their structural polypeptids by SDS-PAGE. Analysis of the viral DNAs with the restriction enzymes HindIII, EcoRI and BamHI gave different fragment patters for the nine isolates. One of them was identified to be a mixture of two other genotypic variants. Similar mobility of som DNA-fragments in each digest suggested, that the isolates are related strains. Homology between the nine isolates was not correlated with their geographical origin.

Key words: DNA restriction endonuclease analysis, geographic variants, *Lymantria dispar*, nuclear polyhedrosis virus, SDS-PAGE

INTRODUCTION

The gypsy moth, *Lymantria dispar*, is a serious pest mainly of cork-oaks in Spain and Italy, cultivated for the production of cork. Several NPVs, wich can be considered as potential control agent were isolated from dead larvae of *Lymantria dispar*, which had been collected from different geographical areas. Screening programmes were performed to select virus strains with increased virulence. Due to the high selectivity of nuclear polyhedrosis viruses (Baculoviridae) for their hosts, these viruses are potential agents for the control of insect pests in agriculture and forestry. For safe and proper use of these viruses in insect pest control, identification of the viruses is recommended.

In general, virus identification and characterization can be achieved by serology,

by biological parameters (morphology, LC50, host range), and by biochemical comparison of polyhedral and/or viral proteins and restriction endonuclease analysis for viral DNAs.

The investigations aimed to identify several virus isolates originated from different areas by byochemical characterization: **i)** Comparison of polyhedral and viral proteins by SDS-PAGE; **ii)** Restriction endonuclease analysis of viral DNAs.

MATERIAL AND METHODS

Origin of virus isolates

Eight virus isolates were obtained from deceased gypsy moth larvae wich were collected in Italy and in Spain and propagated in the institute «Stazione Sperimentale Del Sughero», at Tempio Pausania (Sardinia), by A. Magnoler. One isolate, also obtained from A. Magnoler, originated from a virus production of *Lymantria dispar* NPV (Gypcheck) of Dr. Lewis from USA (table 1). The *Lymantria monacha* isolate originated from Switzerland and was propagated in Darmstadt and Göttingen in Germany (ALTENKIRCH et al., 1986).

Table 1.- Place of origin of each of the nine geographic isolates of *Lymantria dispar.*

Number of virus isolates	Original designation	Origin
1	7311 (Mag)	Italy (Aragogoi) (SS)
2	7405 (Mag)	Italy (Sole) (SS)
3	7409 (Mag)	Italy (Giana Piana)
4	74 (Rup)	Spain (Toledo)
5	840 (Rup)	Spain
6	4218 (Rup)	Spain
7	5135 (Rup)	Spain
8	Con-6006 (Lew)	USA (Hamden, CT)
9	8301 (Mag)	Italy (Tempio)

Virus purification

Before the virus isolates were used for SDS-PAGE or DNA-restriction analysis the polyhedra were purified by isopycnic centrifugation on 50 to 60 % (w/w) linear sucrose gradients in Tris / HCL buffer pH 8 for 2 h by 10000 rpm (Beckman, Rotor JS,-13). The inclusion body bands at approximately 57 % (w/w) sucrose were collected. The harvested bands were washed three times with distilled water by

centrifugation at 17000 rpm for 20 min (Beckman, rotor Ja 20). The virus pellets were resuspended in small amount of Tris /HCL buffer (pH 8) and stored at -18 °C.

Isolation of viral DNA

Virions were released from purified virus particles after incubation with 0.05 M Na_2CO_3, pH10-11, for 30 min at 37 °C. Viral DNA were then liberated from virions after treatment with 1 % SDS and incubation for 60 min at 37 °C. The DNA was extracted twice with phenol saturated with TE buffer (10 mM Tris; 1 mM EDTA pH 8) once with phenol/chloroform (1:1) and once with chloroform/isoamyl alcohol (24:1). The DNA was extensively dialysed against TE and used for DNA analysis.

Restriction-endonuclease analysis

The viral DNA was digested for 4 h at 37 °C with restriction endonucleases EcoRI, BamHi and HindIII (GIBCO/BRL Life Technologies Inc.). The digests were stopped by addition of loading buffer (20 % ficoll; 1 % bromophenol blue; 0.1 M EDTA) and analysed on a horizontal slab gel of 0.6 % agarose. Electrophorensis was carried out at room temperature at 2 V/cm for 16 h. Gels were stained in ethidium bromide (1µg/ml) for 30 min. The stained DNA bands were visualized by fluorscence under a UV light source and photographed using a dark yellow filter. The molecular weight of the viral DNA fragments was determined on slab gels by comparison with the Hind III digest of λ-DNA with known molecular weight.

Sodium-dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Samples of the different virus isolates were treated for SDS-PAGE using the method described by Maskos and Miltenburger, (1981). For better differentiation not only whole polyhedra but also isolated nucleocapsids were analysed.

1. SDS-PAGE of wole polyhedra of *Lymantria dispar* and *Lymantria monacha* NPVs. For this, purified polyhedra of each virus isolate were boiled in sample buffer (20 % sucrose; 10 % 2-mercaptoethanol; 4 % (w/v) SDS; 0.02 % bromophenol blue). Polypeptides were separated by discontinuous SDS-PAGE in a vertical slab gel apparatus using a 5 % stacking and a 11,5 % separating gel. Electrophoresis were carried out for 4.5 h at a constant current of 60 mA. The gels were stained for 1 h in a solution of Coomassie Brilliant Blue. For the determination of the molecular weight of the polypeptides, sandard proteins (Electrophoresis Calibration Kit; Pharmacia) were used.

2. SDS- PAGE of polyhedral and viral polypeptides of *Lymantria dispar* NPVs. Virions were released from polyhedra by solubilization of purified viruses in 0.1 M Na_2CO_3; 0.17 M NaCl; 0.01 M EDTA, pH 10.9 for 80 min by room temperature. The polyhedral proteins were separated from virions by centrifugation on 1.17 to 1.26 g sucrose per ml gradient at 24000 rpm (Hitachi; Rotor RPS 40T) for 60 min. The dissolved polyhedral proteins remained on the gradient wherease the virions were separated in a few bands of different molecular weight (depending on the number of nucleocapsids). The polyhedral protein was collected and precipitated with 0.1 N HCl. After sedimentation (24000 rpm; 25 min) of the precipitate, the pellet was

treated with sample buffer for the SDS-PAGE. Virions were collected at 1.2 to 1.25 g sucrose per ml and sedimentated by 24000 rpm (Hitachi; Rotr RP 65T) for 30 min. The pellet was also treated with sample buffer and used for SDS- PAGE.

RESULTS

Restriction endonuclease analysis of DNA

The DNA purified from each virus isolate of *Lymantria dispar* (1-9) was digested with BamHI, EcoRI and HindIII. The fragments generated by these enzymes are shown for each virus isolate in the following figures 1 to 3. The RE profiles within the nine DNA preparations indicated no conformity for each digest. Differences in number and size of the yelded fragments were visible in all preparations. Nevertheless, a few bands within the DNA profiles showed similar mobilities in each digest, wich were marked in the figures by arrows.

The HindIII-digest of the nine virus isolates yelded 11 to 20 fragments. Five of

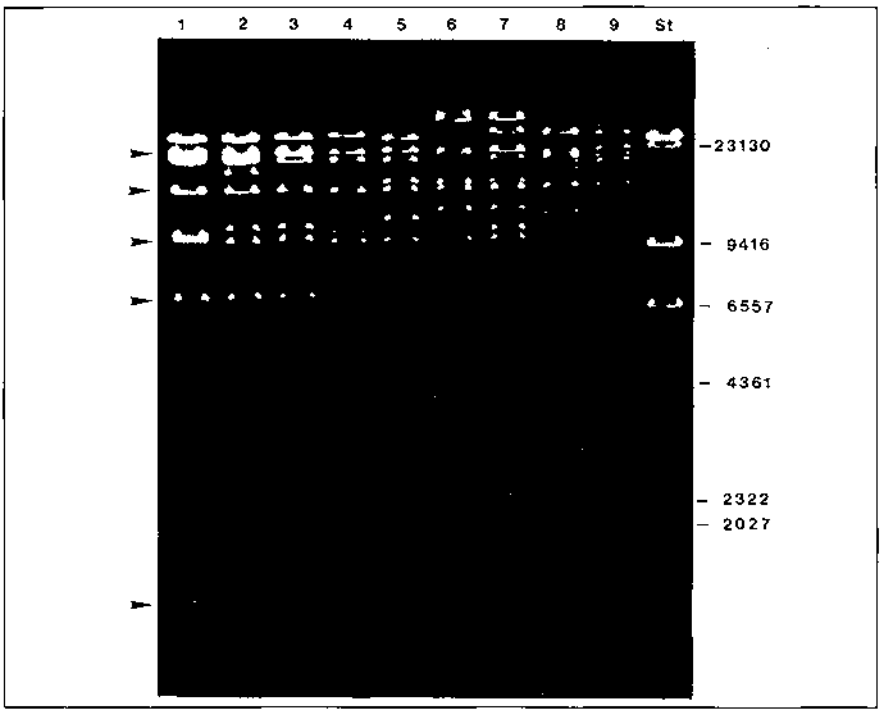

Figure 1.- Restriction endonuclease profile of nine purified virus isolates (lane 1 to 9). Samples were digested with HindIII digest of λEcoRI molecular size makers are given at the right (st). The arrows indicate the comigrating fragments in each DNA profile.

the obtained fragments in size of 21.8 kbp, 15.2 kbp, 7.0 kbp, 2.2 kbp, and one fragment below 2.2 kbp appeared commonly in the Hind III-profil of all the nine isolates.

The BamHI-digest shown in Fig. 2 gave 8 to 13 fragments, whereby 5 fragments comigrated in each profil. The molecular size of these fragments were estimated at 34.5 kbp, 13.9 kbp, 6.6 kbp, 3.3 kbp, and 2.1 kbp.

The EcoRI-digest resulting in 10 to 19 fragments, showed 6 bands in size of 20.0 kbp, 16.8 kbp, 4.5 kbp, 3.0 kbp, 1.8 kbp and 0.9 kbp, which were common in each DNA-pattern of the 9 isolates.

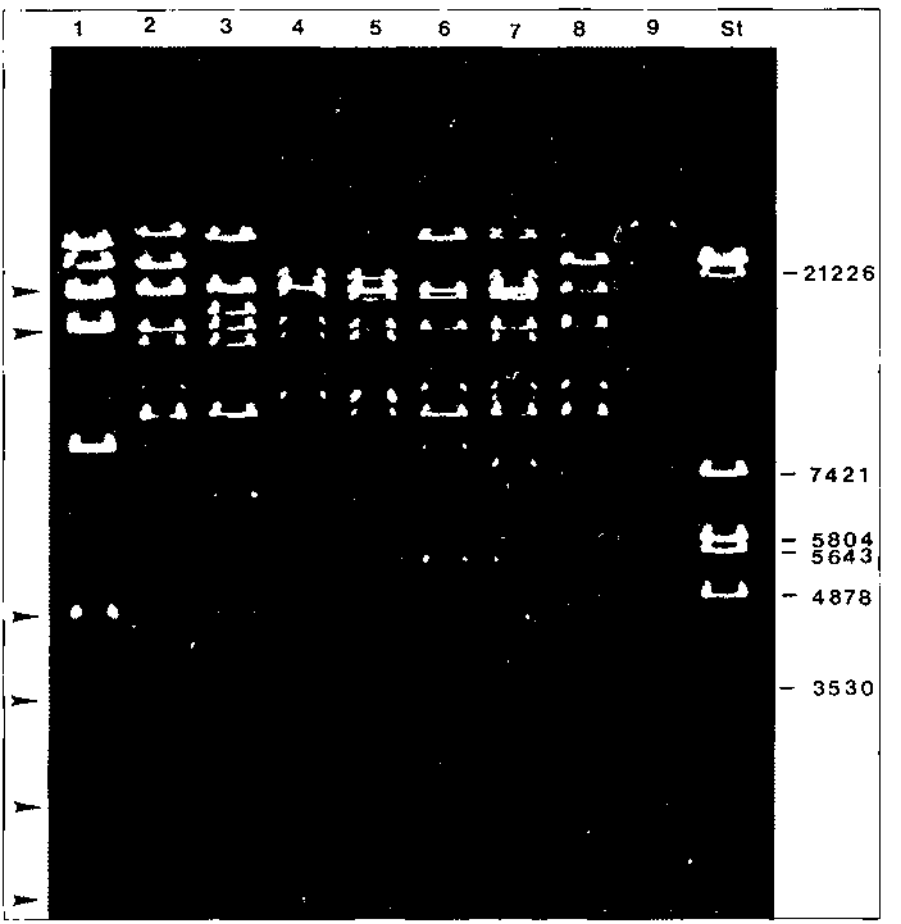

Figure 2.- Restriction enzyme fragments of nine virus isolate DNA digested with EcoRI (lane 1 to 9) were fractionated on 0.6% agarose gel. The λEcoRI molecular size markers are given at the right (St.) The arrows indicate the comigrating fragments in each DNA profile.

The results obtained from DNA analysis of *Lymantria dispar* NPV showed similarities as well as differences in number and size of the fragments.

The genom of all nine isolates could be distinguished when digested with HindIII, EcoRI, and BamHI, whereby one isolate from Spain (5135-E) was identified to be a mixture of two other genotypic variants from Spain (74-E and 4218-E).

In order to see whether closely related viruses can be distinguished from the *L. dispar* NPV, the DNA of a nuclear polyhedrosis virus isolated from *Lymantria monacha* was also characterized by restriction endonuclease analysis. In Fig. 4, the HindIII-profil of the *L. monacha* NPV was compared with the HindIII-profil of the *L. Dispar*

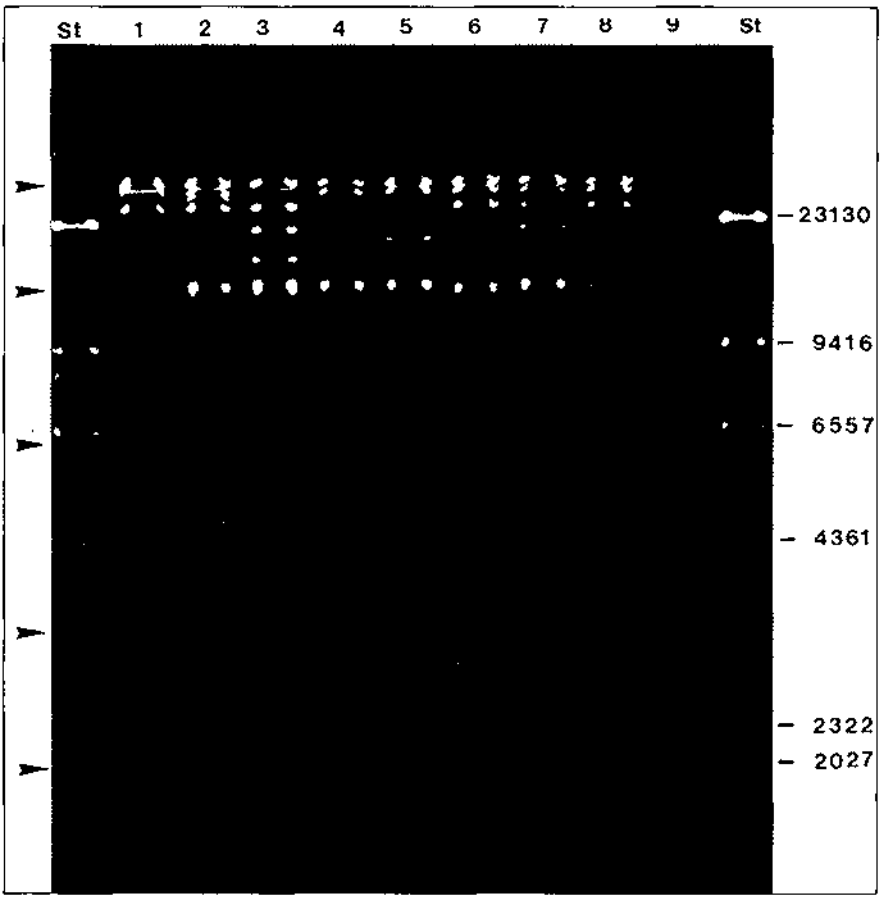

Figure 3.- Agarose gel (0,6%) electrophoresis of DNAs after digestion with endonuclease BamHI (lane 1to 9). The right and left lane indicates HindIII fragments of λDNA as molecular size standard. The arrows indicate the comigrating fragments in each DNA profile.

(7311-I). Comparison of the two profils showed conformity in only one fragment of 15.4 kbp (see arrow).

SDS-PAGE of proteins of *Lymantria dispar*

Samples of different virus isolates from *Lymantria dispar* were treated for SDS-PAGE using the method described above. The SDS-PAGE pattern of the whole protein of the virus isolate (1-8) are shown in figure 5. In all cases no differences were observed in number and mobility of the protein bands. The isolate numbered 9 (not represented in figure 5) was identical to the other ones.

Individual SDS-PAGE analysis of polyhedral and viral polypeptides resulted also in no differentiation between the nine virus isolates. Viral proteins were separated in 9 counterpart polypeptides (Fig. 6) with molecular weights ranging from 32.0 to 80.0 kdalton.

Comparison of the PIB proteins of the isolates (2-9) electrophoresed in SDS-PAGE (Fig. 7) showed that 10 polypeptides are present with molecular weights between 13.0 and 32.0 kdalton. The protein pattern of the isolate (1), not represented

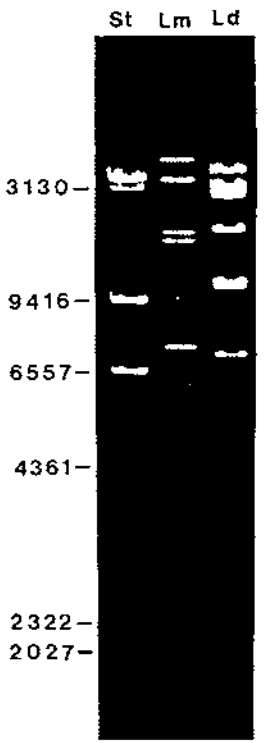

Figure 4.- Comparison of the HindIII-profile of *Lymantria monacha* NPV and *Lymantria dispar* NPV (isolate 7311-I). St characterize the HindIII fragments of λDNA. The aroows marks the DNA band with the same mobility.

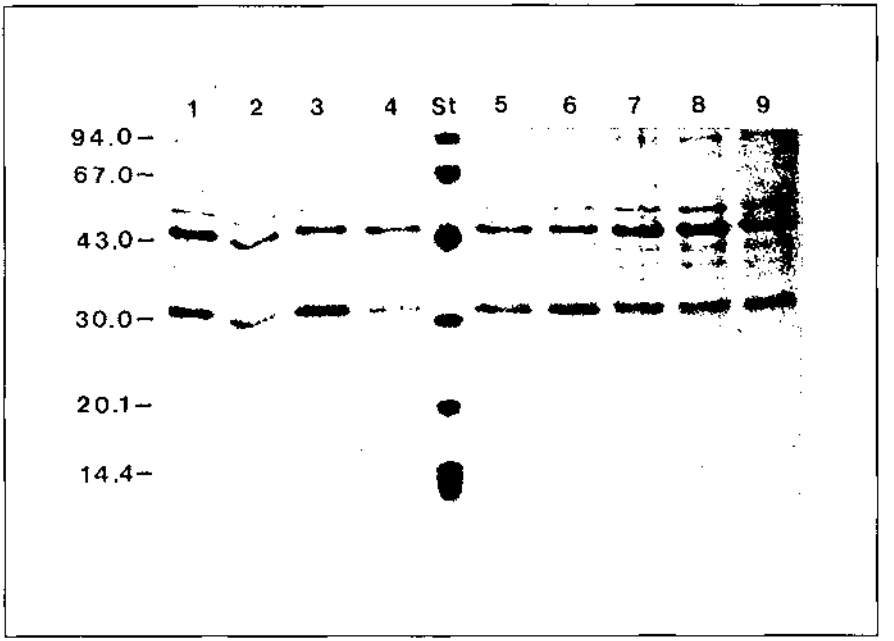

Figure 5.- SDS-PAGE of viral polypeptides of the nine virus isolates from *Lymantria dispar*. The molecular weights of the polypeptides are given in kdalton.

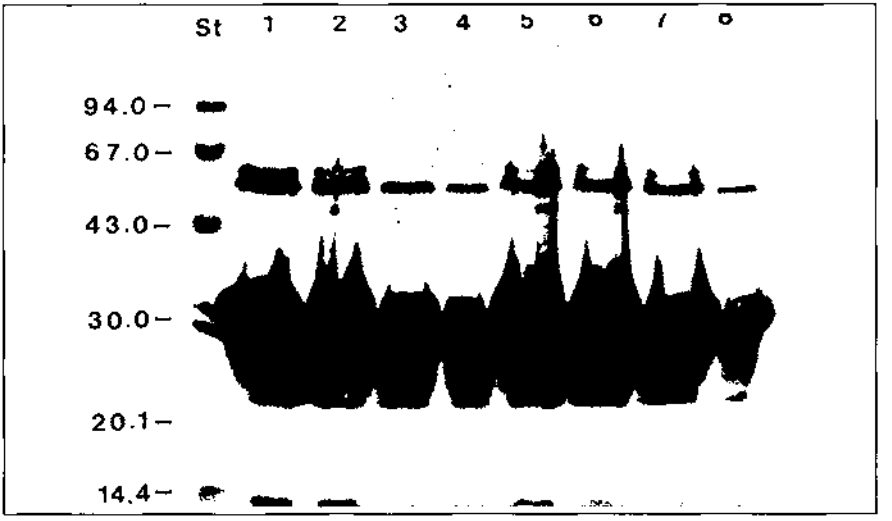

Figure 6.- SDS-PAGE of viral polypeptides of the nine virus isolates from *Lymantria dispar*. The molecular weights of polypeptides are given in kdalton.

isolate in figure 7, gave the same result. The yielded multiple polypeptide bands with lower molecular weight indicate the presence of an endogenous protease with proteolytic degradation activity of major polypeptides after alkaline dissolution. Nevertheless, there was no detectable difference between the polyhedral proteins obtained from the nine virus isolates.

Comparison of protein SDS-PAGE of *L.dispar* and *L.monacha* NPV

In order to see whether other related viruses can be distinguished from *Lymantria dispar* NPV, samples of two NPV isolates from *Lymantria monacha* were also treated for SDS-PAGE.

The SDS-PAGE pattern of the whole virus protein of these two viruses are shown in figure 8. The resulting protein pattern shows agreements as well as differences in number and mobility of the present polypeptides. In spite of the close relatedness of the two virus types it is possible to distinguish between them on the basis of differences in their protein pattern.

DISCUSSION

Identification of baculoviruses isolated from host insect of *Lymantria dispar* collected in the field but originating from different geographical areas showed no differences in polyhedral and viral plypeptide pattern when characterized by SDS-PAGE.

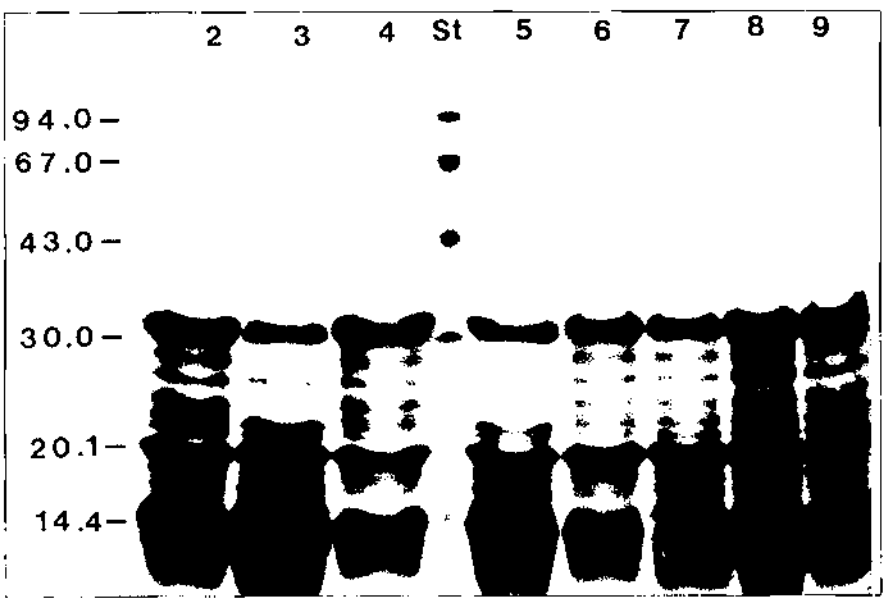

Figure 7.-Comparison of the polyhedral polypeptides of *L. dispar* isolates 2-9.

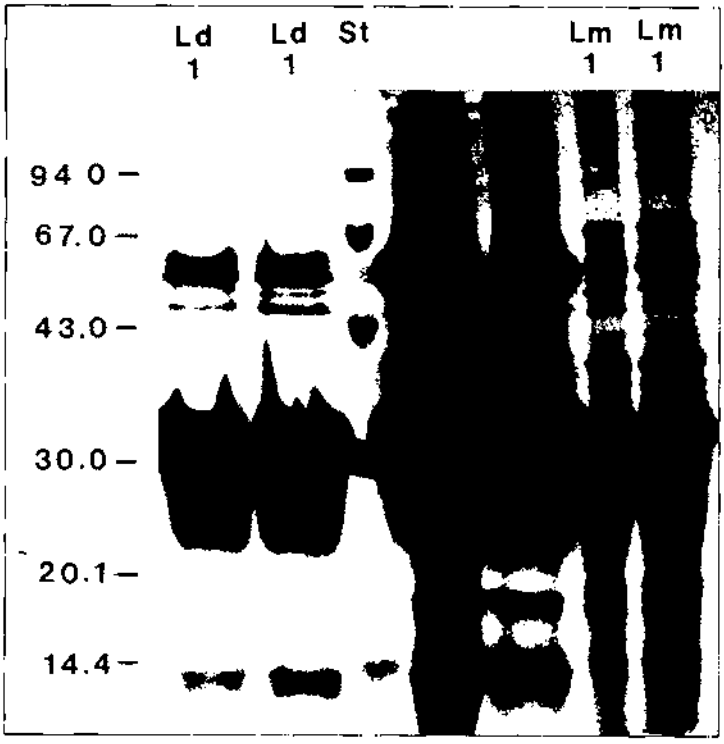

Figure 8.- Comparison of SDS-PAGE pattern of the whole virus proteins from *Lymantria monacha* and *Lymantria dispar* (Gypchek). The molecular weights of the standard proteins are given in Kdalton.

The comparison of this protein pattern with the polypeptides obtained from *Lymantria monacha* NPV, a closely related virus type, shows agreements as cell as differences in number and mobility of the present polypeptides. Comparative studies from ZETHNER et al. (1978) also indicated, that two nuclear polyhedrosis viruses, one from *Lymantria monacha* and one from *L. dispar*, are partially related to each other, but not identical.

The caracterization of the DNA by restriciton endonuclease analysis (RE) on the other hand showed that each virus isolate consists of a distinct genotype on the basis of RE profiles for BamHI, EcoRI and HindIII. In this context, VLAK & GRÖNER reported 1980, that two *Mamestra brassicae* NPVs found in two geographical areas, could be distinguished by RE analysis for their DNA. Similar results were obtained by DNA characterization of eight geographic isolates of granulosis viruses (Vickers et al., 1991).

This results demonstrate, that DNA restriction endonuclease analysis is able to identify and to distindguish geographical variants, event to reveal genetical heterogeneity within one isolate indicated by the presence of submolar bands

(Smith & Crook, 1988). This submolar fragments were mainly visible in the EcoRI digests suggesting that more than one genotype was present in the *L. dispar* NPV isolates. One isolate was identified to be a mixture of two other genotypic variants. This genotypic heterogeneity appears to be widespread among field isolates of NPVs (Lee & Miller, 1978; Knell & Summers, 1981).

The results obtained from biochemical characterization studies seggest that the field isolates recovered from different geographical areas are closely related. To determine the degree of homology hybridization methods would be needed.

References

KNELL, J. D. & SUMMERS, M. D. 1981. Investigation of geneticheterogeneity in wild isolates of *Spodoptera frugiperda* nuclear polyhedrosis virus by restriction endonyclesae analysis of plaque purified variants. *Virology* 112, 190-197.

MASKOS, C. B. & MILTENBURGER, H. G. 1981. SDS-PAGE comparative studies on the polyhedras and viral polypedptides fo the NPV of *Mamestra brassicae*, *Autographa californica* and *Lymantria dispar*. *J. Invertebr. Pathol.* 37, 174-180.

SMITH, I. R. & CROOK, N. E. 1988. In vivo isolation of baculovirus genotypes. *Virology* 166, 240-244 .

VICKERS, J. M.; CORY, J. S. & ENTSWISTLE, P. F. 1991. DNA characterization of eight geographic isolates of granulosis virus form the potato tuber moth (*Phtorimae operculella*) (Lepidoptera, Gelechiidae). *J. Invertebr. Pathol.* 57, 334-342.

VLAK, J. M. & GRÖNER, A. 1980. Identification of two nuclear polhedrosis viruses from the cabbage moth, *Mamestra brassicae* (Lepidoptera, Noctuidae). *J. Invertebr. Pathol.* 35, 269-278.

ZETHNER, O.; BROWN, D. A. & HARRAP, K. A. 1979. Comparative studies on the nuclear polygedrosis virus of *Lymantria monacha* and *Lymantria dispar*. *J. Invertebr. Pathol.* 34, 178-183.