SELECTION OF ACTIVE STRAINS OF THE GYPSY MOTH NUCLEARPOLYHEDROSIS VIRUS

M. Shapiro and E. Dougherty

Shapiro-Agricultural Research Service-U.S. Department of Agriculture, Otis Methods Development Center, Otis, ANGB, MA 02542

Dougherty-Agricultural Research Service-U.S. Department of Agriculture, Insect Pathology Laboratory, Bldg. 011A, BARC-West, Beltsville, MD 20705

The gypsy moth Lymantria dispar (linnaeus) has grown in economic importance as an insect pest over the past 75 years. From a localized infestation of a small geographical area of New England, the gypsy moth has spread to such an extent that is now found over much of the United States. Control measures are varied, but effective biological control is needed to control pest populations, especially in such sensitive areas as parks, residential areas, and municipal watersheds.

A nuclear polyhedrosis virus (NPV) of the gypsy moth has been registered for control purposes; however, efficacy is variable and borderline in control situations. Natural epizootics of the virus are extremely effective in destroying natural populations. The disparity in efficacy between natural virus outbreaks and artificial application of laboratory produced gypsy moth virus presents an obvious research problem for the selection of a more 'potent' strain of the virus. The approaches that we have taken involve: (1) in vitro strain selection; (2) evaluation of naturally occurring geographical isolates; (3) in vivo passage through L. dispar; and (4) in vitro and in vivo mutagenesis.

In vitro strain selection

Cell lines of ovarian origin were obtained by Dr. R. H. Goodwin of the Insect Pathology Laboratory (BARC-USDA). Using the IPLB-652Y cell line, a plaque assay was developed (Fig. 1), which not only allows for quantification of non-occuled virus but also allows for studying the replication and genetics of LdMNPV. Using the plaque assay, 13 plaque isolates were made and their virulence compared to the LDP-67 strain (U.S. Forest Service, Hamden, CT) (Table 1). No one isolate was more virulent than the standard (LDP-67); however, a few isolates (5-7D,14-4C) were close in activity. There was a wide range in activity with three samples giving no activity greater than 4250 times an LD_{50} for the LDP-67 isolate.

Isolation of DNA from the plaque isolates listed in Table 1 and digestion with restriction endonucleases (REN) showed that the twelve isolates could be separated into a least five groups based upon the profile of REN digests. Differences in the digestion profiles can be observed in Figure 2, which shows an EcoRI digest of the plaque isolates. Although many bands are common to the plaque isolates, obvious differences do exist. A summation of differences found among the twelve isolates using the restriction enzymes SAL I, BGL II, BAM HI, HInD III, and EcoRI are presented in Table 2. Utilization of the five enzymes tests produced five different groupings of isolates with common REN patterns. Use of additional restriction enzymes could potentially show further dissimilarities.

IPLB-652 cells Initial Density 10^5/cells/cm^2
↓
2 hr attachment remove medium
↓
Virus Dilutions made in KC52B medium
0.8 ml/25cm^2 flask
↓
1 hr incubation - gentle rocking
↓
Remove virus innoculum
↓
Add 3.0 ml KC52B medium containing 0.5% agarose
↓
Incubate 7 days @ 28°C
↓
Stain cell sheet with 0.01% neutral red
↓
Count plaques under an indirect light

Figure 1.--A plaque assay for Lymantria dispar MNPV using the IPLB-652 cell line.

Thus, comparison of the five groupings of plaque isolates having common patterns (Table 2) and virulence (Table 1) show that genotypic variants of L. dispar NPV do display differential virulence. These studies have shown that there is a diversity of genomic material within isolate LDP-67.

Naturally-occurring geographical NPV isolates

Differences in biological activity can be detected among geographical isolates of the same NPV species (Ossowski 1960, Smirnoff 1961, Chauthani et al. 1968, Shapiro and Ignoffo 1970), including the gypsy moth NPV (Magnoler 1970, Rollinson and Lewis 1973, Vasiljevic and Injac 1973). Our objective was to identify the most active isolates by comparing NPV isolates from North America, Europe, and Asia (Shapiro et al. 1984). Eighteen isolates of L. dispar NPV were harvested from both laboratory and field-collected larvae, and were compared to a Connecticut standard (LDP-67) is standardized bio-assays (Shapiro and Bell 1981). LD_{50}s varied from 1.7×10^3 polyhedral inclusion bodies (PIB)ml ($=0.34 \times 10^0$ PIB/mm^2 of diet surface) to 5000×10^3 PIB/ml ($=1 \times 10^3$ PIB/mm^2), a difference of about 2940-fold (Table 3). The most active NPV isolates generally originated from North America, the least active isolate originated from Asia (Japan). In the highest activity group, one

Table I. Bioassay of L. dispar Multiple Embedded Nuclear Polyhedrosis Virus Plaque Isolates

Plaque Isolate	LD_{50} (PIB/ml X 10^4)	Relative Efficiency (Percent)	Plaque Isolate	LD_{50} (PIB/ml X 10^4)	Relative Efficiency (Percent)
LDP-67	1.77	100.0			
5-7D	5.83	30.3	9-12	66.00	2.6
14-4c	6.57	26.9	7-109	125.35	1.4
12-4b	7.97	22.2	6-9a	7500	.02
11-13c	8.60	20.5	14-10b	7500	.02
13-7a	9.00	19.7	15-10c	7500	.02
10-13a	10.47	16.9			
16-13a	14.33	12.3			
8-12	15.00	11.8			

Figure 2

ECO RI DIGEST OF PLAQUE PURIFIED ISOLATES
OF THE LDP-67 STRAIN OF MLd NPV

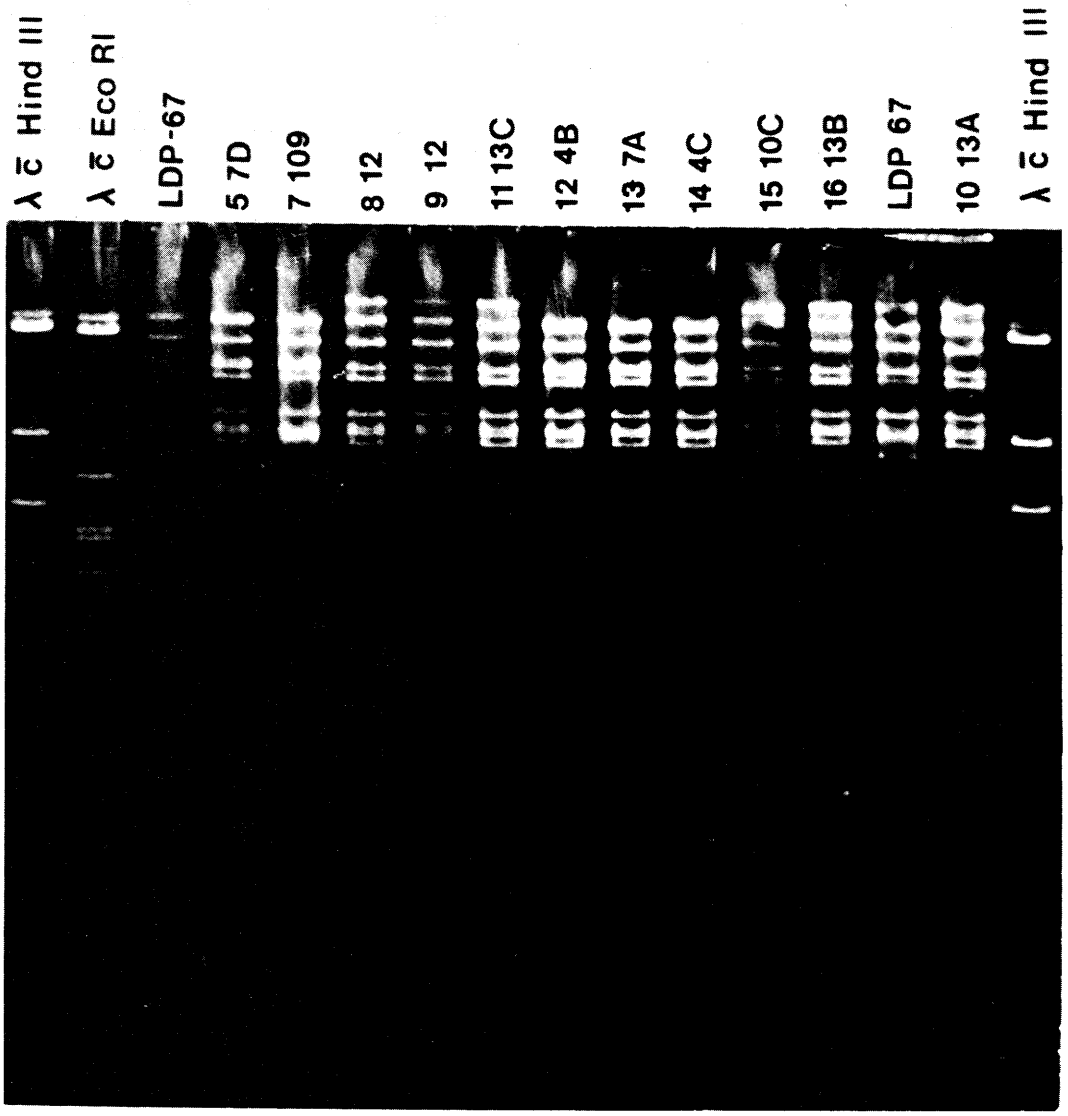


Table 2. LdMNPV Plaque Isolates: Summary of Grouping of Isolates According to REN Profiles

		Sal I	Bgl II	Bam HI	Hind III	Eco RI
LDP-67	(WT)	X	Y	Z	A	A
1D	5-7d	X	Y	Z	B	A
1F	7-109	X	Y	Z	A	A
1g	8-12	X	Y	Z	A	B
1h	9-12	X	Y	Z	-	B
1j	11-13c	X	Y	Z	A	B
1k	12-4b	X	Y	Z	B	A
1l	13-7a	X	Y	Z	B	A
1N	14-4c	X	Y	Z	B	A
1o	15-10c	X	Y	Z	-	B
1p	16-13b	X	Y	Z	A	B
1q	6-9a	X	Y	Z	A	C
1i	10-13a	X	Y	Z	C	B

Plaque Isolates with Common REN Patterns

aa	ab	ba	ac	cb
LDP-67	1g	1d	1q	2i
1f	1j	1k		
	1p	1l		
		1n		

Table 3.--Comparatige biological activities of L. dispar NPV isolates

NPV ISOLATE TESTED	LC_{50} (95% CL) PIB/ml x 10^3
QUEBEC 1	1.7(1.1-2.6)
ABINGTON, MASS.	1.8(1.0-3.3)
QUEBEC 2	2.4(0.8-6.4)
DIGHTON, MASS.	3.0(2.4-5.8)
BALD EAGLE, PA.	3.7(2.9-4.7)
LDP-67(CT STD)	4.8(4.0-5.8)
HOUGHTON POND, MASS.	5.0(3.3-7.6)
NEW HAMPSHIRE	6.8(5.3-8.8)
MIDDLEPORT, PA.	8.0(6.3-10.6)
ROMANIA	13.1(10.6-16.2)
SANDWICH, MASS.	21.0(14.9-29.6)
BRIDGEWATER, MASS.	21.4(13.4-33.7)
ORANGE, MASS.	27.3(17.3-43.5)
SERBIA-YUGOSLAVIA	28.8(23.3-35.6)
USSR	31.3(26.6-36.9)
SLOVANIA-YUGOSLAVIA	57.1(22.3-133.8)
DALMATIA-YUGOSLAVIA	59.3(48.7-72.2)
SPAIN	78.8
JAPAN	Greater than 5000

(From Shapiro et al. 1984).

isolate came from the United States (Abington, MA), and two came from Canada (Quebec 1 and 2). In the next activity level, one isolate came from Dighton, MA and one came from Bald Eagle, PA. Since these five isolates were significantly more active than the CT standard, they might be potential candidates for use as microbial control agents. The European isolates were in the sixth to eleventh activity levels. At a concentration of $5000x10^3$ PIB/ml, the Japanese NPV caused only 16% larval mortality, whereas lower concentrations were not lethal (Shapiro et al. 1984).

Virus was purified from pooled cadavers from these experiments and the respectove viral genomes were purified and digested with a variety of restriction enzymes. As in the previous instance, there is a variation in bands of restriction fragments among the various geographical isolates, showing a great heterogeneity both intra-strain and inter-strain among L. dispar NPVs. Using a nick translated probe of the genome of LDP-67 to hybridize against a Southern blot of Figure 3, it was found that the greatest homology between LDP-67 and foreign isolates occurred among North American isolates, intermediate homology occurred with European isolates, and no homology occurred with the Asian isolate. Thus, reports stating the gypsy moth is an evolving species whose North American members now have different physiology, behavior, etc. could also pertain to the gypsy moth NPVs.

In vivo passage through Lymantria dispar

Eight virus isolates (USA-LDP-67; ROMANIA, SPAIN, USSR, YUGOSLAVIA-DALMATIA, SERBIA, SLOVENIA) were selected serial passage through second-stage L. dispar larvae. While the activity of the American isolate did not change during the four passages, activities of all other isolates increased significantly during the second passage (Table 4). Moreover, the activity of the Japanese isolate increased ca. 1000-fold during the passages. In general, activities became stabilized from the second to the fourth passage and were quite similar to that of LDP-67. The experiment was terminated after the fourth passage, since it was assumed, based upon the behavior of LDP-67, that further increase in activity would be minimal. While serial passage resulted in greater activity (see also Veber 1972, Shapiro and Ignoffo 1970), these isolates were still significantly less ($P<.05$) than the most active North American isolates (Quebec 1, Quebec 2, and Abington, MA) (Shapiro et al. 1984).

Since the NPV isolate from Abington, MA had greater biological activity ($P<.05$) than the LDP-67 standard, a concentrated effort was made to characterize this isolate, in terms of activity. The Abington isolate was serially passed, along with the LDP-67 isolate, at LD_{50} values. As was seen previously, serial passage of LDP-67 did not result in any change in activity, which indicated the stability of the isolate. Surprisingly, the activity of AB decreased during early passage through second-stage L. dispa larvae, but the NPV was still more active than the LDP-67 standard (Fig. 4). During subsequent passage of AB, however, activity increased to the original value, and remained stable during the last three passages. A sample of AB was then forwarded to the U.S. Forest Service Laboratory, Hamden, CT for testing. The high activity of this isolate was confirmed (W. Rollinson, personal communication) and large amounts of this isolate will be produced for subsequent field-testing.

Mutagenesis

In addition to identifying and monitoring natural genetic variation, artificially induced genetic variation is also being investigated. Over ten years ago, Reichelderfer and Benton (1973) reported that four treatments of an NPV (Spodoptera frugiperda) with 3-methylcholanthrene resulted in a nine-fold increase in virulence. A significant decrease in the Lt_{50} was observed during bioassays comparing treated and untreated virus. Moreover, similar results were observed with 1,2,3,4-dibenzanthracene, 5-bromodeoxyuridine,

Figure 3

GEOGRAPHICAL ISOLATES OF
LYMANTRIA DISPAR NUCLEAR POLYHEDROSIS VIRUS
DIGESTED WITH BAM HI

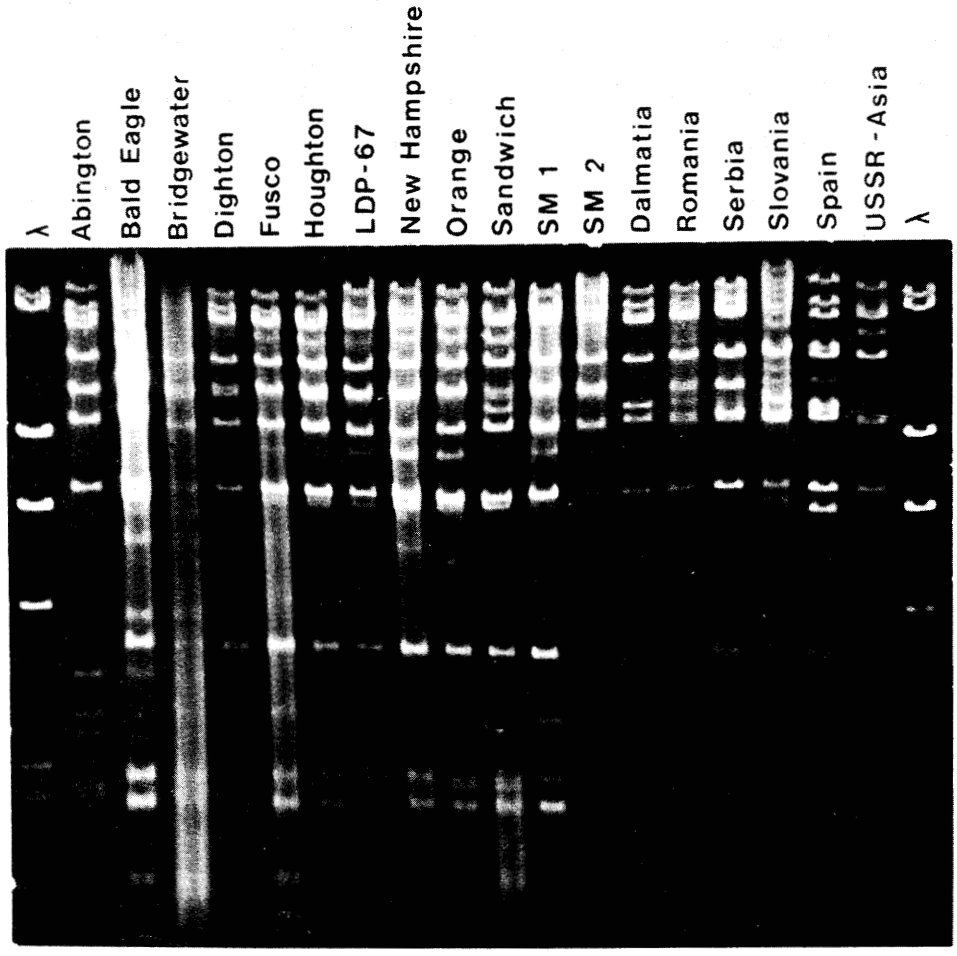
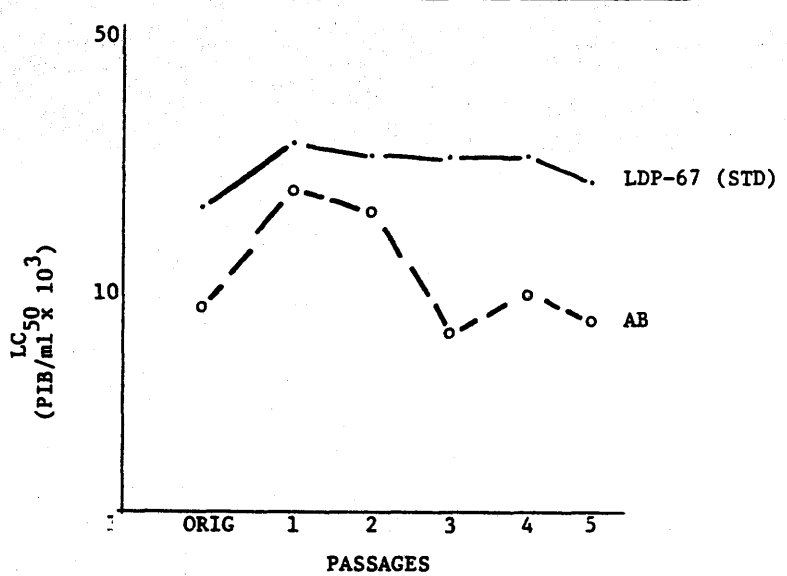


Table 4.- BIOLOGICAL ACTIVITIES OF NPV ISOLATES SERIALLY PASSED THROUGH L. dispar LARVAE

NPV ISOLATES TESTED	LC_{50}(95%CL)(PIB/ml x 10^3) AT PASSAGE 1	4
LDP-67 (STD)	6.26(3.98-9.58)	6.06(4.61-7/97)
SPAIN	94.6(41.2-200)	5.60(3.36-9.16)
ROMANIA	14.1(9.05-21.7)	5.82(2.42-13.2)
DALMATIA-YUGO	59.6(29.6-117.2)	6.10(4.30-8.60)
SLOVANIA-YUGO	57.6(25.9-120)	8.00(6.09-10.5)
SERBIA-YUGO	33.6(17.3-63.2)	9.95(6.35-15.4)
USSR	29.8(18.8-44.8)	5.87(4.47-7.67)
JAPAN	5000+	9.53(5.98-15.4)

(From Shapiro et al. 1984).

Fig. 4 SERIAL PASSAGE OF ABINGTON, MA NPV ISOLATE



N.B. ORIGINAL AB NPV ISOLATE WAS ALSO BIOASSAYED AT EACH PASSAGE, AND THE LC_{50} VALUES WERE 8.0, 8.3, 7.2, 9.6, AND 9.1 (MEAN VALUE = 8.44 x 10^2 PIB/ML).

and 5-iododeoxyuridine. In the case of the alfalfa looper (Autographa californica) NPV, however, no significant effects were noted with 5-bromodeoxyuridine or n-methyl-n'-nitro-n-nitrosoguanidine (McClintock 1980) in the cabbage looper, bollworm, tobacco budworm, or fall armyworm. A mutant of the alfalfa looper NPV (=HOB) was obtained in vitro after treatment with 2-aminopurine, which possessed increased virulence (Wood et al. 1981). In our investigations, in vitro experiments are now in progress uisng the mutagen 2-aminopurine. These experiments, along with co-infection studies with homologous and heterologous virions and genomes (co-transfection) to generate non-genetically engineered recombinant viruses should produce genomes having artificially or non-naturally occurring genetic variation. In addition, in vivo studies are now in progress using such mutagens as bromodeoxyuridine, bromodeoxycytidine, 2-sminopurine, ethyl methyl sulfonate, hydroxylamine, acriflavin, proflavin, and hydrogen peroxide, among others. More virulent strains are hoped for from these studies.

The empirical search for an array of genes which will produce a more virulent strain of LdMNPV has been or is now undergoing all classical and standard methods used to produce virulent strains of virus both in animal virology and in insect virology. Failure to produce such a strain does not imply that these methods be abandoned; however, the use of new strategies developed from the Biology revolution of the last decade should be considered. If lessons are to be learned from other viruses which have been manipulated by these methods, then a large amount of fundamental work such as described is necessary to intelligently modify current Lymantria dispa nuclear polyhedrosis viruses.

Literature Cited

Chauthani, A. R., D. Claussen, and C. S. Rehnborg. 1968. Dosage-mortality data on a nuclear-polyhedrosis virus of the bollworm, Heliothis zea. J. Invertebr. Pathol. 12:335-338.

Magnoler, A. 1970. Susceptibility of gypsy moth larvae to Lymantria sp. nuclear and cytoplasmic viruses. Entomophaga 15:407-412.

McClintock, J. T. 1980. Treatment of a nuclear polyhedrosis virus of Autographa californica with chemical mutagens and determination of changes in virulence in four lepidopterous hosts. M. S. Thesis, U. of Maryland, 72 pp.

Ossowski, L. L. J. 1960. Variation in virulence of a wattle bagworm virus. J. Insect Pathol., 2:34-43.

Reichelderfer, C. F., and C. V. Benton. 1973. The effect of 3-methylcholanthrene treatment on the virulence of a nuclear polyhedrosis virus of Spodoptera frugiperda. J. Invertebr. Pathol. 22:38-41.

Rollinson, W. D., and F. B. Lewis. 1973 Susceptibility of gypsy moth larvae to Lymantria spp. nuclear and cytoplasmic polyhedrosis viruses. Plant. Prot. 24. No. 124-125: 163-168.

Shapiro, M., and C. M. Ignoffo. 1970. Nucleopolyhedrosis of Heliothis: activity of isolates from Heliothis zea. J. Invertebr. Pathol. 16:107-111.

Shapiro, M., J. L. Robertson, M. G. Injac, K. Katagiri, and R. A. Bell. 1984. Comparative infectivities of gypsy moth (Lepidoptera: Lymentriidae) nucleopolyhedrosis virus isolates from North America, Europe, and Asia. J. Econ. Entomol. 7:153-156.

Smirnoff, W. A. 1961. A virus disease of Neodiprion swainei Middleton. J. Insect Pathol. 3:29-46.

Vasiljevic, L., and M. Injac. 1973. A study gypsy moth viruses originating from different geographical regions. Plant Prot. 24. No. 124-125: 169-186.

Veber, J. 1962. Virulence of an insect virus increased by repeated passage. Colloq. Int. Pathol. Insectes, Paris. 3:403-405.

Wood, H. A., P. R. Hughes, L. B. Johnston, and W. H. R. Langridge. 1981. Increased virulence of Autographa californica nuclear polyhedrosis virus by mutagenesis. J. Invertebr. Pathol. 38:236-241.