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# Geographic isolates of *Lymantria dispar multiple nucleopolyhedrovirus*: Genome sequence analysis and pathogenicity against European and Asian gypsy moth strains

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ABSTRACT

Isolates of the baculovirus species *Lymantria dispar multiple nucleopolyhedrovirus* have been formulated and applied to suppress outbreaks of the gypsy moth, *L. dispar*. To evaluate the genetic diversity in this species at the genomic level, the genomes of three isolates from Massachusetts, USA (LdMNPV-Abba624), Spain (LdMNPV-3054), and Japan (LdMNPV-3041) were sequenced and compared with four previously determined LdMNPV genome sequences. The LdMNPV genome sequences were collinear and contained the same *homologous repeats* (hrs) and clusters of *baculovirus repeat orf* (*bro*) gene family members in the same relative positions in their genomes, although sequence identities in these regions were low. Of 146 non-*bro* ORFs annotated in the genome of the representative isolate LdMNPV 5-6, 135 ORFs were found in every other LdMNPV genome, including the 37 core genes of *Baculoviridae* and other genes conserved in genus *Alphabaculovirus*. Phylogenetic inference with an alignment of the core gene nucleotide sequences grouped isolates 3041 (Japan) and 2161 (Korea) separately from a cluster containing isolates from Europe, North America, and Russia. To examine phenotypic diversity, bioassays were carried out with a selection of isolates against neonate larvae from three European gypsy moth (*Lymantria dispar dispar*) and three Asian gypsy moth (*Lymantria dispar asiatica* and *Lymantria dispar japonica*) colonies. LdMNPV isolates 2161 (Korea), 3029 (Russia), and 3041 (Japan) exhibited a greater degree of pathogenicity against all *L. dispar* strains than LdMNPV from a sample of Gypchek. This study provides additional information on the genetic diversity of LdMNPV isolates and their activity against the Asian gypsy moth, a potential invasive pest of North American trees and forests.

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## 1. Introduction

*Lymantria dispar multiple nucleopolyhedrovirus* is a species in the genus *Alphabaculovirus* of the insect virus family *Baculoviridae* (Herniou et al., 2012). Viruses of this family form virions consisting of a single double-stranded circular DNA genome contained in an enveloped, rod-shaped capsid (Harrison and Hoover, 2012). Baculovirus DNA replication and progeny virus assembly occur in the nucleus of the host. Initially, a type of progeny virion, referred to as budded virus (BV), is produced when nucleocapsids exit the nucleus and bud through the host plasma membrane, acquiring an envelope in the process. Later during the replication cycle, nucleocapsids are enveloped, either singly or in bunches, within the nucleus. The resulting virions, known as occlusion-derived virus (ODV), are assembled into a paracrystalline matrix composed of a single viral protein, polyhedrin, which is synthesized at very high levels in infected cells. This occlusion process results in the formation of occlusion bodies (OBs; also known as polyhedra) which contain multiple virions (Slack and Arif, 2007).

OBs serve to transmit viral infection horizontally within a host population (Fuxa, 2004). Larval stages of insects from orders Lepidoptera, Diptera, and Hymenoptera become infected when they ingest OBs. The OB protein matrix dissolves in the host gut, and the liberated ODV enter the host midgut epithelial cells. From this point, progeny BV secreted from infected cells serve to disseminate infection within the host. Baculovirus infections generally result in the death of the host. Cadavers of baculovirus-killed hosts break down due to the action of viral-encoded degradative enzymes, and progeny OBs are released into the environment. The OB matrix

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confers a degree of environmental persistence to the virions occluded within, which allows for the retention of infectivity until another larva ingests the OBs and renews the cycle.

Baculovirus OBs have been produced and formulated for use as safe, ecologically and environmentally friendly biopesticides (Moscardi, 1999). Isolates of *Lymantria dispar multiple nucleopolyhedrovirus* have been used in this way to control outbreaks of its host, *L. dispar*, the gypsy moth, a pest of trees and forests (Solter and Hajek, 2009). Populations of the subspecies *Lymantria dispar dispar* (common name: European gypsy moth) are found in Europe and in North America, where it is an invasive pest. *L. dispar dispar* has been spreading throughout the Northeast corner of the USA and adjacent areas in Canada since its introduction in Massachusetts, USA in the late 1860s (Pogue and Schaefer, 2007). Its dispersal has been slow, likely due to efforts to hinder its spread and to the inability of adult females to fly. There are also two subspecies, *Lymantria dispar asiatica* and *Lymantria dispar japonica*, which are part of the Asian gypsy moth complex (Pogue and Schaefer, 2007). Populations of *L. dispar asiatica* are found in Russia east of the Ural Mountains, northern China, and the Korean peninsula, while *L. dispar japonica* populations are found in Japan. The Asian gypsy moth subspecies are currently not established in North America, but Asian gypsy moths have been detected and eradicated in the United States on at least 23 occasions between 1991 and 2014 (USDA/APHIS/PPQ, 2015). The Asian gypsy moth poses a serious invasive threat to North American trees and forests due to the broader plant host range of the larvae and the ability of adult females to fly.

The isolate Lymantria dispar multiple nucleopolyhedrovirus LDP-67 (LdMNPV LDP-67) has formed the basis for Gypchek, a biocontrol product currently produced by the USDA Forest Service and Sylvar Technologies Inc. (Canada) for use against outbreaks of gypsy moth in North America (Reardon et al., 2012). Other LdMNPV isolates have been tested for activity against gypsy moth populations in North America and Asia (Duan et al., 2012; Lewis et al., 1984; Narang et al., 2001; Shapiro et al., 1984). An isolate has also been used to develop the product Virin-ENSh for use against gypsy moth in the former Soviet Union (Alyoshina, 1980). Results from comparative bioassays with LdMNPV isolates have raised the possibility that there may be differences in the susceptibilities of European and Asian gypsy moth larvae to LdMNPV infection (Ebling et al., 2004). Differences in pathogenicity may affect the capacity of current formulations of Gypchek to control invading populations of Asian gypsy moth.

Basic research on the molecular biology and genetics of LdMNPV has been carried out primarily with strain LDP-67 or clonal isolates derived from it (McClintock et al., 1986; Slavicek and Podgwaite, 1992; Slavicek et al., 1995, 1992). The entire genome sequence of the plaque isolate LdMNPV 5-6 was determined by Sanger dideoxy sequencing in 1999 (Kuzio et al., 1999). The advent of next-generation sequencing technologies have facilitated the sequencing of baculovirus genomes, and genome sequences have now been determined for isolates LdMNPV-2161 from South Korea (Harrison et al., 2014); LdMNPV-27 from Western Siberia, Russia (Kabilov et al., 2015); and LdMNPV-3029, a sample from the biopesticide Virin-ENSh (Harrison and Rowley, 2015). The data from these sequences, along with data from partial sequencing of the *lef-8* gene from several additional LdMNPV isolates in a USDA insect virus collection, suggest that viruses from the *L. dispar* populations in Europe and North America have diverged from viruses found in Asian *L. dispar* populations (Harrison et al., 2014).

In this study, three additional LdMNPV genomes – one from a plaque isolate derived from a Massachusetts (USA) population, an isolate from Spain, and an isolate from Japan – were completely sequenced in order to amass more information on the genetic diversity of this group of viruses at the genomic level and confirm the grouping of LdMNPV isolates into European/North American and Asian assemblages. The pathogenicities of the isolates in Gypchek and Virin-ENSh and Asian LdMNPV isolates were compared in bioassays with European and Asian gypsy moth colonies to obtain information on LdMNPV phenotypic diversity and to evaluate the control potential of different LdMNPV isolates against European and Asian gypsy moth populations.

## 2. Materials and methods

### 2.1. Virus isolates and insects

LdMNPV-Ab-a624 is a plaque isolate obtained by plating hemolymph of larvae infected with an Abington, MA LdMNPV sample on the cell line IPLB-LdEIta (Lynn et al., 1993). Other LdMNPV isolates featured in this study are from a USDA Agricultural Research Service insect virus collection maintained in Beltsville, MD, and include LdMNPV-3049, a sample of Gypchek deposited in September 1997; LdMNPV-2161, an isolate collected in South Korea by D. K. Reed (Pemberton et al., 1993) and deposited September 28, 1993; LdMNPV-3029, a sample of Virin-ENSh; LdMNPV-3041, collected in Japan; and LdMNPV-3054, an isolate from Spain deposited November 26, 1980 (Harrison et al., 2014). Virus isolates were grown in 3rd and 4th instar larvae of the New Jersey Standard Strain of *L. dispar*, reared from eggs obtained from the USDA APHIS rearing facility in Otis AFB, MA on *L. dispar*-specific diet from Southland Products (Lake Village, AR) at 28 °C on a 14:10 light:dark cycle.

Bioassays were carried out with a selection of Asian and European gypsy moth strains maintained at the USDA Forest Service Northern Research Station Quarantine Facility in Ansonia, CT. These strains included *L. dispar japonica* strain JN from Nagoya, Japan; two *L. dispar asiatica* strains, including strain RM from Mineralni, Primorski in Far East Russia; strain RB from Bellyk, Krasnoyarsk in Siberia, Russia; and three *L. dispar dispar* strains, including strain LJ from Juodkrante, Kuzsin Nezijos in Lithuania; strain KG from Kavála, Macedonia in Greece; and strain UC from Bethany, New Haven County in Connecticut, USA. These strains and their maintenance are described in Keena et al. (2008). Each generation is produced from 100 randomly-selected egg masses to maintain genetic diversity. The identities of these colonies have been confirmed in a recent barcoding study (Chen et al., 2016).

### 2.2. Genomic DNA preparation and 454 sequencing

For each virus isolate to be sequenced, genomic DNA was isolated and sequenced as previously described (Harrison and Lynn, 2007; Harrison et al., 2014). Sequencing reads from a Roche 454 GS Junior instrument were sorted and assembled using the SeqMan NGEN V3.0 assembler program (Lasergene; DNASTAR, Inc., Madison, WI) with default parameters. Gaps were closed and regions with ambiguous sequences or unusual features were resolved or confirmed by PCR amplification and Sanger dideoxy sequencing. The Lasergene SeqManPro (version 9) sequence editor was used to prepare the final contigs of the consensus genome sequences. Sequence coverage and GenBank accession numbers for each isolate are listed in Table 1.

Open reading frames (ORFs) were manually annotated for each genome by selecting ORFs of at least 50 codons that did not overlap adjacent ORFs by >75 bp. ORFs were also selected for which annotated homologues existed in other baculovirus genomes, including other genomes of LdMNPV. BLASTp queries were carried out to determine the relatedness of predicted amino acid sequences to those of LdMNPV 5-6 and other baculoviruses. Intergenic *homologous repeat* (*hr*) sequences were identified by searching the