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Pathology and genome sequence of a Lymantria dispar multiple nucleopolyhedrovirus (LdMNPV) isolate from Heilongjiang, China

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ABSTRACT

The pathogenicity and genome sequence of isolate LdMNPV-HrB of the gypsy moth alphabaculovirus, Lymantria dispar multiple nucleopolyhedrovirus from Harbin, Heilongjiang, China, were determined. A stock of this virus from one passage through the gypsy moth New Jersey Standard Strain (LdMNPV-HrB-NJSS) exhibited 6.2- to 11.9-fold greater pathogenicity against larvae from a Harbin colony of *L. dispar asiatica* than both Gypchek and a Massachusetts, USA LdMNPV isolate (LdMNPV-Ab-a624). Sequence determination and phylogenetic analysis of LdMNPV-HrB and LdMNPV-HrB-NJSS revealed that these isolates were most similar to other east Asian LdMNPV isolates with 98.8% genome sequence identity and formed a group with the east Asian LdMNPV isolates which was separate from groups of isolates from Russia, Europe, and USA.

1. Introduction

The gypsy moth (*Lymantria dispar* L., Lepidoptera: Erebidae) is a worldwide pest of trees and forests. Currently, three sub-species are recognized (Pogue and Schaefer, 2007): *Lymantria dispar dispar* L., which is native to Europe; *L. dispar asiatica* Vnukovskij, which is found in Asia east of the Ural Mountains, China, and Korea; and *L. dispar japonica* Motschulsky, found in Japan. The latter two subspecies are collectively referred to as the Asian gypsy moth (AGM), and comprise a biotype which is distinguished from *L. dispar dispar* (European gypsy moth, or EGM) by the ability of AGM female moths to fly, while EGM female moths are flightless (Keena et al., 2008).

The European gypsy moth was introduced into the United States at Medford, MA in 1868. Since then it has spread from the northeast corner of the USA, extending south to Virginia and west to Wisconsin and Illinois and adjacent regions in Canada (https://www.aphis.usda.gov/plant_health/plant_pest_info/gypsy_moth/downloads/gypmoth.pdf). The costs from outbreaks of this pest in terms of economic damage and control measures are considerable (Aukema et al., 2011; Bradshaw et al., 2016). An invasion of North America by AGM is expected to be even more damaging and costly, due to the flight capability of female AGM moths and the possibility that AGM populations will better adapt to some North American trees than the current invasive EGM populations (Keena and Richards, 2020). AGM has been detected in the USA 24 times between 1991 and 2015 (USDA/APHIS/PPQ, 2016). In addition, 580 specimens of AGM egg masses were intercepted at 20 different US ports of entry during 2019, a 630% increase relative to 2018 (Veira et al., 2019).

One of the control measures used to manage outbreaks of *L. dispar dispar* in the USA is a product, Gypchek, consisting of a formulation of the Lymantria dispar multiple nucleopolyhedrovirus (LdMNPV) (Reardon et al., 2016), an alphabaculovirus in family *Baculoviridae* (Harrison et al., 2018). The LdMNPV isolate used for Gypchek originates from a population of EGM in Connecticut, USA (Reardon et al., 2016). Different isolates from different EGM and AGM host populations exhibit different levels of pathogenicity against EGM and AGM larvae. Data from some studies suggest that some isolates may be more effective than Gypchek against populations of AGM (Duan et al., 2011, 2012; Ebling et al., 2004; Harrison et al., 2016; Podgwaite et al., 2013), though not all studies indicate a significant difference among isolates towards different host populations (Martemyanov et al., 2017).

We previously reported a preliminary characterization of an LdMNPV isolate, LdMNPV-HrB, from a gypsy moth population located in Harbin, China (Harrison et al., 2014). In this study, we assessed the

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Table 1
Concentration-mortality response ($LC_{50} \times 10^4$ in OBs/mL, with 95% confidence limits).

LdMNPV isolates	Strains of *L. dispar*[a]					
	L. dispar dispar	*L. dispar asiatica*				*L. dispar japonica*
	UC (Connecticut, USA)	SK (Gangwon, Korea)	CJ (Beijng, China)	CN (Liaoning, China)	CR (Heilongjiang, China)	JI (Honshu, Japan)
Gypchek	6.1 (4.7–7.9) ***ab***	2.0 (1.2–3.0) ***a***	4.9 (3.3–7.3) ***a***	3.3 (1.9–5.3) ***a***	23.0 (10.3–118.6) ***a***	2.5 (1.1–4.7) ***ab***
Ab-a624	4.3 (2.6–6.9) ***a***	1.9 (1.2–2.8) ***a***	2.4 (1.2–4.2) ***b***	3.5 (1.5–7.4) ***a***	44.0 (17.2–453.3) ***a***	2.8 (2.2–3.5) ***a***
3029	12.3 (6.7–28.4) ***c***	2.0 (1.2–3.1) ***a***	1.2 (0.8–1.6) ***b***	5.8 (3.9–8.8) ***b***	4.4 (3.3–5.8) ***b***	3.4 (0.7–10.8) ***b***
HrB-NJSS	7.5 (5.5–10.5) ***b***	2.2 (1.1–3.7) ***a***	1.5 (0.7–2.7) ***b***	5.3 (4.0–6.9) ***b***	3.7 (2.8–4.9) ***b***	1.9 (1.0–3.0) ***a***

[a] For each *L. dispar* strain, different letters denote statistically significant differences among LC50 values of isolates.

pathogenicity of LdMNPV-HrB against larvae of different EGM and AGM colonies and determined the genome sequence of this isolate and its relationships to other LdMNPV isolates.

2. Materials and methods

2.1. Virus isolates and insects

LdMNPV isolates used in bioassays included LdMNPV-Ab-a624 (Lynn et al., 1993), LdMNPV-3029, a sample of the biopesticide Virin-ENSh (Harrison et al., 2014; Harrison and Rowley, 2015); a sample of Gypchek obtained from Sylvar Technologies, Inc. (Harrison et al., 2016); and LdMNPV-HrB-NJSS, from a single passage of LdMNPV-HrB through the New Jersey Standard Strain (NJSS) *L. dispar* larvae. Colonies of *L. dispar* were maintained at the USDA Forest Service Northern Research Station Quarantine Facility in Ansonia, CT as previously described (Keena, 2016). The colonies used in this study included UC (from Connecticut, USA; (Keena et al., 2008)); SK (South Korea; (Keena, 2016); JI (Honshu, Japan; (Keena, 2016); and CB (Beijing), CL (Liaoning), and CR (Harbin), from three different provinces in China (Chen et al., 2015).

2.2. Bioassays

Occlusion body (OB) stocks for use in bioassays were prepared from cadavers of infected 4th instar larvae that had been reared from the same shipment of NJSS *L. dispar* eggs obtained from the APHIS Buzzards Bay Laboratory in MA, USA. Droplet feeding bioassays were carried out and scored as previously described (Harrison et al., 2014, 2016) with neonate larvae of UC, SK, CB, CR, CL, and JI colonies. Southland Products gypsy moth diet was supplemented with either 0.03 g/L (UC) or 0.11 g/L (AGM colonies) of ferric citrate. The LC_{50} values were calculated from the data of three replicate bioassays and compared by lethal dose ratio test using Polo-Plus 2.0 (Robertson et al., 2007). Slopes of probit lines and heterogeneities (x^2/n) are shown in Supplementary Table 1.

2.3. Genome determination and phylogeny

LdMNPV-HrB viral DNA was isolated from the original sample of OBs that had been harvested from virus-killed Harbin (CR) larvae (Harrison et al., 2014), and also from a second preparation of OBs (LdMNPV-HrB-NJSS) harvested from 4th-instar NJSS larvae that were infected with LdMNPV-HrB, using previously described procedures (Harrison et al., 2014). The DNA from these two preparations were sequenced, and genomic contigs were assembled and annotated as previously described (Harrison et al., 2016).

Available LdMNPV genome sequences (Supplementary Table 2) were aligned with each other and the genome of the Lymantria xylina nucleopolyhedrovirus (LyxyNPV-5) (Nai et al., 2010) by MAFFT as implemented in Lasergene MegAlign Pro v 17 (DNAStar). Phylogenetic relationships were inferred from this alignment by maximum likelihood using MEGA X (Kumar et al., 2018) with the Tamura 3-parameter model and a discrete gamma distribution for rate variation among sites while allowing some sites to be invariable. Nucleotide positions with representation in less than 50% of the taxa were excluded from the analysis. Reliability of the tree was evaluated with 500 bootstrap iterations.

3. Results

3.1. Relative pathogenicity of LdMNPV-HrB-NJSS and other isolates to EGM and AGM larvae

In bioassays with *L. dispar* colonies UC, CJ, CN, and JI, LC_{50}s among the four LdMNPV isolates tested often exhibited statistically significant differences but with a low degree of variation which ranged from 1.2- to 4.1-fold (Table 1). In contrast, the Gypchek and Ab-a624 viruses exhibited LC_{50}s against the CR colony from Harbin, Heilongjiang, China that were 5.2- to 11.9-fold higher than the HrB-NJSS isolate from the same population or the 3029 isolate. Gypchek and Ab-a624 LC_{50}s against CR were also approximately 10X higher than LC_{50}s of Gypchek and Ab-a624 against the other five colonies.

3.2. Harbin LdMNPV genome sequences and relationships with other LdMNPV isolates

Because bioassays were carried out with a stock of LdMNPV-HrB that had been passaged through NJSS larvae, genome sequences for both the original virus stock (LdMNPV-HrB) from colony CR (Harbin) larvae and from LdMNPV-HrB after a single passage through NJSS larvae (LdMNPV-HrB-NJSS) were determined and compared to evaluate the impact of a single passage of an AGM-derived virus isolate through EGM larvae on the genome sequence.

The LdMNPV-HrB genome was 162,246 bp with a nucleotide composition of 57.4% G + C, which are within the ranges of genome sizes (159,089–164,746 bp) and nucleotide compositions (57.3–57.5%) of other LdMNPV isolates (Supplementary Table 2). Differences in sizes of LdMNPV genomes were often attributable to deletions unique to specific isolates (Harrison et al., 2016), including deletions of *baculovirus-repeated ORF* (*bro*) sequences and the *viral enhancing factor-1* (*vef*-1) gene (Martemyanov et al., 2017). The features annotated in the LdMNPV-HrB sequence include 174 ORFs (including *vef-1*) and 12 homologous regions (*hrs*) (Supplementary Table 3), with 16 *bros*, 147 homologs of ORFs annotated in the exemplar isolate LdMNPV-5/6, and 17 ORFs with homologs annotated in other LdMNPV isolates and LyxyNPV-5. The LdMNPV-HrB ORFs conserved in the exemplar isolate LdMNPV-5/6 encoded amino acid sequences with identities ranging from 65.2% (ORF63, *chaB1*) to 100% (ORF1, *polh*; ORF18, *odv-e18*; ORF40, *p10*; ORF72, *chitinase*; ORF86, *ac75*; ORF87, *ac76*; ORF90, *gp41*; ORF97, *p18*; ORF100, *odv-e28*; ORF104, *p40*; ORF105, *p12*; ORF108, *pif-7*; ORF125; ORF156; *ctl-1*; ORF161; ORF166) (Supplementary Table 3).

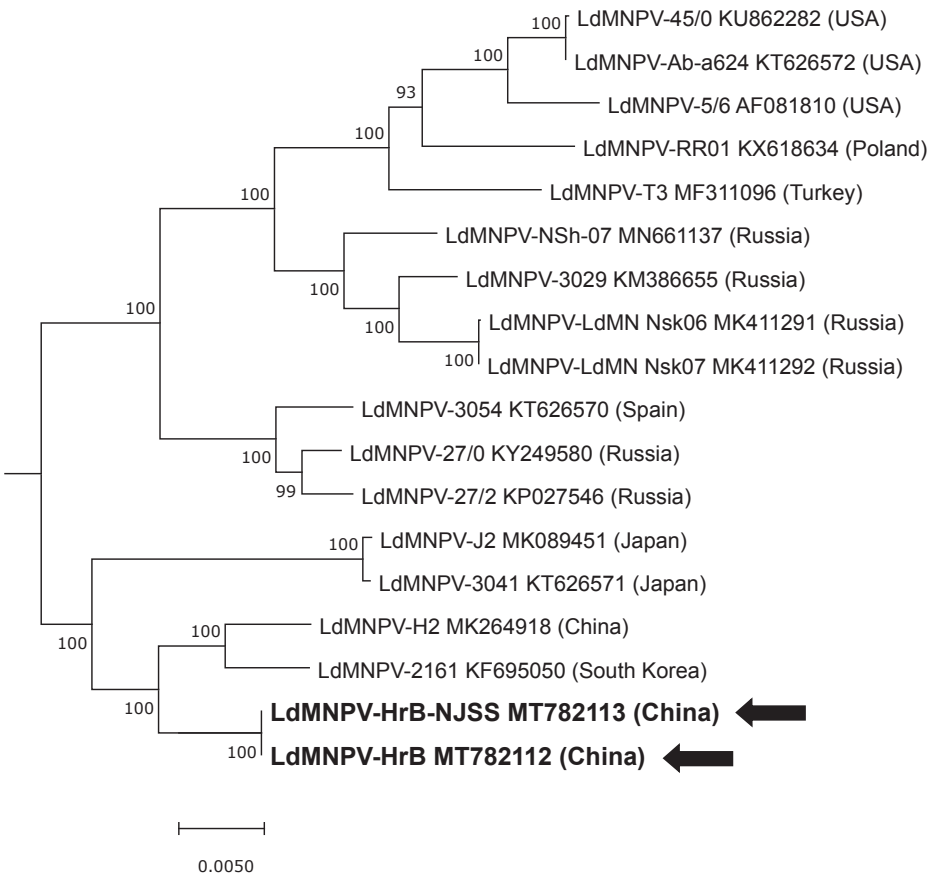

Fig. 1. Phylogenetic inference of relationships among isolates of LdMNPV based on whole-genome nucleotide sequence alignments. A maximum-likelihood phylogram of isolates is shown with GenBank accession numbers and host geographic origin. LdMNPV-HrB and LdMNPV-HrB-NJSS are indicated in bold type with arrows. The related alphabaculovirus LyxyNPV-5 from *Lymantria xylina* (Nai et al., 2010) was included as an outgroup (not shown). Bootstrap values are displayed at each node. Pairwise % nucleotide sequence identities between LdMNPV-HrB and the other LdMNPV genome sequences ranged from 97.1% (with LdMNPV-RR01) to 98.8% (with LdMNPV-2161 and LdMNPV-H2).

Of the most divergent ORF sequences, many are *bro* genes and others do not have a known or identifiable function.

Comparison of the LdMNPV-HrB and -HrB-NJSS genome sequences revealed that the consensus sequences of these two isolates differed by two indels: a 57-bp insertion in LdMNPV-HrB-NJSS running from nt 6657–6766, within ORF4 (*mucin-like*); and a 21-bp deletion present in LdMNPV-HrB-NJSS of nt 85,589–85,609 of the LdMNPV-HrB sequence in ORF93 (*vp91*). The two sequences possess the same set of ORFs (Supplementary Table 3). Numerous polymorphisms (both SNPs and indels) were present in the reads of both genome sequences in frequencies that often differed by >10% (data not shown).

Alignment of LdMNPV-HrB and -Hrb-NJSS with complete genome sequences of other LdMNPV isolates confirmed an extensive degree of collinearity among the genomes of these viriuses, with pairwise % sequence identities ranging from 97.1% (with LdMNPV-RR01) to 98.8% (with LdMNPV-2161 and LdMNPV-H2). The HrB isolates were grouped with isolates LdMNPV-H2 from China and LdMNPV-2161 from South Korea in a phylogeny inferred from the genome alignment (Fig. 1). These viruses were part of a larger clade that included isolates from Japan. Isolates from Russia, Europe, and the USA formed a separate clade.

4. Discussion

The significantly higher pathogenicity of LdMNPV-HrB-NJSS and LdMNPV-3029 towards larvae of the CR colony relative to Gypchek suggests that these isolates may be more effective for controlling invading populations of AGM from northern China. The Gypchek virus prep from Sylvar Technologies that was used in this study did not exhibit the same comparatively reduced pathogenicity that was observed for a Gypchek sample, isolate LdMNPV-3049, that had been deposited in the USDA-Beltsville virus collection in 1997 (Harrison et al., 2016). The differences between these two Gypchek preparations may be due to differences in genotypic compositions (Podgwaite et al., 2010). To our knowledge, there is no published data on the potencies of different samples of the same baculovirus isolate that would assist in understanding our results with different samples of Gypchek.

Phylogenetic analysis of LdMNPV isolates from genome sequence alignments indicate that the geographic location of a host population exerts more influence on the divergence of, and relationships among, viral isolates than the sub-species or biotype of the host. Isolates from east Asian *L. dispar asiatica* populations in China and Korea were grouped together with isolates from Japanese populations, at least one of which (JI) is *L. dispar japonica*, and separately from isolates deriving from other *L. dispar asiatica* populations in Russia. In addition, the clade containing USA and European isolates from *L. dispar dispar* populations were placed in a larger group with Russian isolates from *L. dispar asiatica* populations. This same trend was observed with respect to host plant utilization by different populations of gypsy moth (Keena and Richards, 2020), indicating that both gypsy moth baculovirus genetics and gypsy moth host plant adaptation traits are locally adapted and not fixed to gypsy moth subspecies or biotype.

The results in this study suggest that different isolates should be considered when developing formulations for controlling invading populations of AGM. Further research is needed to identify the specific variables in both host populations and virus isolates that influence pathogenicity.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2020.107495.

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