Journal of Invertebrate Pathology 203 (2024) 108063

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Genomics of alphabaculovirus isolates infecting *Mamestra* species from North America and Eurasia

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ARTICLE INFO

Keywords:
Alphabaculovirus
Mamestra
Genomics
Geographic isolates

ABSTRACT

Whole genome sequencing and multiplex PCR analysis were used to characterize previously isolated baculovirus isolates from *Mamestra* populations in Eurasia. Although these viruses have been previously described as Mamestra brassicae nucleopolyhedrovirus (MbNPV/MabrNPV), we demonstrate here that these isolates represent strains of the baculovirus species *Alphabaculovirus maconfiguratae* (MacoNPV-A) and *Alphabaculovirus altermaconfiguratae* (*MacoNPV-B*). The MabrNPV-Bu and –Uk isolates had 96% nucleotide (nt) identity to the type isolate MacoNPV-A 90/2 at the whole genome level and in addition contained a *lef-7* homologue which is found in MacoNPV-A but not MacoNPV-B. MabrNPV-Si, -De and –Nl had 96.6, 96.6 and 98.5% nt identity to the type isolate MacoNPV-B 96/2 at the whole genome level, respectively and contained a *helicase-2* homologue. Gene content, synteny and K-2-P *lef-8*, *lef-9* and *polh* analysis also confirmed the presence of both MacoNPV-A and MacoNPV-B isolates in Eurasia. Thus, both these alphabaculovirus species have wide Holarctic distributions in *Mamestra* host species. MacoNPV-A and MacoNPV-B have wide host ranges and in addition we showed that MacoNPV-B isolates trended to higher infectivity for *T. ni* larvae.

1. Introduction

Mamestra brassicae Linnaeus (Lepidoptera: Noctuidae; cabbage moth) is a palearctic species with an extensive Eurasian distribution from the Atlantic to Pacific coasts. It is a polyphagous species having been recorded on at least 70 host plant species but is mainly known as a pest of cruciferous vegetable crops (Ahuja, *et al.*, 2010; Turnock & Carl, 1995). A substantial number of alphabaculovirus strains have been isolated from *M. brassicae* populations (Possee & Kelly, 1988) and indeed one isolate was developed as a viral insecticide under the tradename Mamestrin [NPP, Nagueres, France] for control of the cabbage moth (Erlandson, 2008; Murillo et al., 2001). *Mamestra configurata* Walker is a nearctic species with a broad geographic range in western North America. It is a polyphagous species having been recorded from at least 40 host plant species; however, its major native host plant range likely included members of the *Chenopodiaceae* and the *Brassicaceae*, but it is a major pest on canola and flax crops (Mason et al., 1998). Multiple baculovirus isolates have been derived from *M. configurata* populations from across its geographic range and complete genome sequence analysis identified two *Alphabaculovirus* species, *Alphabaculovirus maconfiguratae* (MacoNPV-A) and *Alphabaculovirus altermaconfiguratae* (MacoNPV-B), isolated from the same host species (Li et al., 2002a,b).

In the *Baculoviridae*, species parameters and demarcation are not well defined but have been framed in terms of host range and specificity, DNA restriction endonuclease profiles, and DNA and protein sequence identities, particularly for a subset of conserved genes (van Oers et al., 2023; Harrison *et al.*, 2019;Wennmann et al., 2018). In general this fits the species definition now utilized by the International Committee for the Taxonomy of Viruses (ICTV) which defines a species as a monophyletic group of MGEs (mobile genetic elements) whose properties can be distinguished from those of other species by multiple criteria (https://ictv.global). As indicated above, a significant number of alphabaculovirus isolates have been described from populations of *Mamestra* species from both Eurasia (*M. brassicae*) and North America (*M. configurata*) (Aruga et al., 1960; Brown et al., 1981; Burgerjon et al., 1975; Erlandson, 1990; Possee & Kelly, 1988; Vlak & Gröner, 1980; Wiegers & Vlak, 1984). The complete genome sequences are available for a number of these isolates, and gene synteny and homology

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https://doi.org/10.1016/j.jip.2024.108063
Received 15 November 2023; Received in revised form 13 January 2024; Accepted 17 January 2024
Available online 28 January 2024

comparisons indicate that two alphabaculovirus species are found in *Mamestra* hosts. Comparative analysis of the complete genome sequences of isolates of MacoNPV-B (Li, L. *et al.*, 2002) and MabrNPV (Choi et al., 2013) and including an isolate from *Helicoverpa armigera* (Tang et al., 2012) indicate that these viruses are members of the same species, *Alphabaculovirus altermaconfiguratae*. The isolates of a second species, *Alphabaculovirus maconfiguratae*, were considered distinct based on a lower (less than 95 % identity) homology of amino acid sequence for homologous open reading frames (ORF) when compared to the MacoNPV-B and MabrNPV ORF sequences. In addition, all MacoNPV-A genomes contain *lef-7* homologues which are absent in MacoNPV-B and MabrNPV/MbNPV isolate genomes (Li, Q. *et al.*, 2001; Li, L. *et al.*, 2002).

Previous biochemical analysis of a number of the Eurasian alphabaculovirus isolates from *M. brassicae* and other hosts suggested that many of these viruses were genetically distinct but related (Brown et al., 1981; Possee & Kelly, 1988; Roseti *et al.*, 2000; Vlak & Gröner, 1980; Wiegers & Vlak, 1984). However, oral bioassay data suggested that these virus isolates had no significant difference in infectivity or virulence for *M. brassicae* or related species (Brown et al., 1981; Possee & Kelly, 1988; Roseti *et al.*, 2000; Vlak & Gröner, 1980; Wiegers & Vlak, 1984). The host range of the MbMNPV Oxford isolate (originally from A. Gröner, Darmstadt, Germany) was extensively tested across four orders on insects (Doyle et al., 1990). Only lepidopteran hosts were susceptible to infection, and of the 66 lepidopteran species tested, 32 were infected by the MbMNPV Oxford isolate and more than 90 % of the susceptible hosts belonged to the family Noctuidae (Doyle et al., 1990). Although the host range of MacoNPV-A and MacoNPV-B has not been extensively tested, MacoNPV-B was shown to be more infectious in *T. ni* and *Spodoptera exigua* than MacoNPV-A (Li, L. *et al.*, 2002). In addition, in North America MacoNPV-A isolates have been isolated from field collected larvae of noctuid species other than *M. configurata*, including: *Lacinobia subjuncta*, *Acetebia fennica* and *Euxoa messoria* (M.A. Erlandson, unpublished data).

We had the opportunity to further characterize, by complete genome sequence analyses, additional alphabaculovirus isolates from putative *M. brassicae* populations from across Eurasia which had been previously characterized by REN analysis (described in van Oers & Vlak, 2007). The availability of these isolates gave us the opportunity to test the hypothesis that there would be a spatial gradient of genotypes over a wide geographic range i.e., the greater the geographic distance between isolate sites of origins the higher the divergence of genotypes would be. The results of the current study indicate that representatives of both MacoNPV-A and MacoNPV-B are found among these Eurasian isolates and there did not seem to be a close correlation between geographic distance and genotype divergence. Based on these results we propose that all these described alphabaculovirus isolates from *Mamestra* populations belong to one of the two formally described and accepted species, *Alphabaculovirus maconfiguratae* (MacoNPV-A) or *Alphabaculovirus altermaconfiguratae* (MacoNPV-B). However, given that both these virus species have relatively broad host ranges and similar infectivity in a relatively wide spectrum of related noctuid species, it may be difficult to determine the host species complex in which each of these two alphabaculovirus species evolved or if in fact they co-evolved in the same host complex.

2. Materials and methods

2.1. Alphabaculovirus isolates

Semi-purified occlusion body (OB) preparations of the following "MbNPV/MabrNPV" isolates: Kyiv, Ukraine (MabrNPV-Uk); Heidelberg, Germany (MabrNPV-De); Split, Croatia (MabrNPV-Yu), Kostinbrod, Bulgaria (MabrNPV-Bu), Novosibirsk, Russia [Siberia] (MabrNPV-Si) and Wageningen, The Netherlands (MabrNPV-Nl) were amplified by infecting *Mamestra brassicae* larvae from a laboratory colony at Laboratory of Virology, Wageningen University.

2.2. Insects

A long-term laboratory culture of *M. configurata*, first established in 1974, was maintained on a semi-synthetic diet and larvae reared at 21 °C, 60 % relative humidity and an 18:6 light/dark photoperiod (Bucher & Bracken, 1976).

A long-term colony of *T. ni*, originally obtained from Dr. A. Keddie (University of Alberta, Edmonton, Alberta, Canada), was maintained on a sterile semi-synthetic diet (Keddie and Volkman, 1985) and the larvae reared at 27 °C, 60 % relative humidity and a 20:4 light/dark photoperiod. Embryonated eggs were routinely surface disinfected by a 3 min dip treatment in 0.6 % commercial bleach and rinsed in sterile ddH_2O.

2.3. Virus DNA purification

Virus DNA was purified from occlusion-derived-virions (ODV) released from approximately 5.0x10^8 occlusion bodies (OB) using previously described methods (Erlandson, 1990). Briefly, the OB suspension was incubated in purification buffer (TE pH7.5, 0.5 %SDS, 0.1 % Triton-X100) at 37 °C for 1 h, filtered through three layers of cheesecloth and the OBs pelleted at 5,000*g* for 15 min. OBs were resuspended in sterile ddH_2O at 2.0x10^8 OB/ml. Approximately 5.0x10^8 OB were mixed with an equal volume of OB dissolution buffer (0.1 M Na_2CO_3; 0.001 M Na_2EDTA; 0.17 NaCl pH 10.8) and incubated for ~ 20 min at RT until OB dissolution was complete as monitored by phase contrast microscopy. The suspension was then neutralized by addition of 1/10th volume of 1 M Tris-HCl, pH7.5. Released ODV were pelleted by centrifugation through a 20 % sucrose cushion in SW28 Beckman rotor at 95,000 *g* for 1 h. The ODV were resuspended in 500ul of TE pH 7.5 and incubated at 60 °C to inactivate nucleases, then incubated in 1.0 % SDS and 5 mg/ml proteinase K at 37 °C for 2 h. Finally, DNA was extracted twice with an equal volume of phenol:chloroform:isoamylalcohol (50:48:2) and once with an equal volume of chloroform:isoamlyalcohol (24:1). The aqueous phase was dialyzed extensively in Slide-A-Lyzer Dialysis Cassettes, 3,500 MWCO – 0.5 mL capacity (Thermo-Scientific) against TE pH 7.5 with 4 changes of 500 mL over 36 h at 4 °C. The DNA was then quantified by absorbance at 260 and 280 nm using a Nanodrop spectrophotometer (Fisher-Thermo). Virus isolate DNA samples were also analyzed by restriction endonuclease digestion of 500 ng of DNA and electrophoresis on 0.7 % agarose gels.

2.4. Species identification using multiplex PCR

A multiplex PCR assay was developed as a rapid method for distinguishing isolates as either MacoNPV-A or MacoNPV-B. As the *hoar* gene sequence is quite variable among field isolates of the same species as well as between NPV species (Le et al., 1997), *hoar* primers specific to MacoNPV-A and MacoNPV-B and producing different size amplicons were designed (Table 1). In addition, all North American isolates of MacoNPV-A contain a *lef-7* homolog which is not present in the MacoNPV-B isolates. MacoNPV-A specific *lef-7* primers were designed to be compatible with *hoar* primers and were used in a multiplex PCR assay (Table 1). The PCR reactions mixtures were as follows: each 25 μL reaction contained 2.5 μL 10X PCR reaction buffer, 2.5 mM $MgCl_2$, 0.4 μM dNTPs, 0.4 μM of each primer, 1 U Taq DNA polymerase (Invitrogen, Burlington, ON, Canada) and approximately 5 ng of DNA template. The PCR reactions were conducted in a BIO-RAD C1000 Thermal Cycler using the following conditions: an initial denaturation step at 94 °C for 3 min; followed by 21 cycles at 94 °C for 30 s, 58 °C for 40 s and 72 °C for 60 s and finally at 72 °C for 5 m.

2.5. DNA sequencing, assembly, annotation and analysis

Virus DNA samples, ~ 500 ng, were sequenced at the National Research Council, Plant Biotechnology Institute (Saskatoon, Saskatchewan, Canada) using Roche 454 FLX-titanium pyrosequencing

Table 1
Primer names, sequence and predicited amplicon size for MacoNPV-A and MacoNPV-B *hoar* and MacoNPV-A lef-7 target seqences.

Name	Sequence	Name	Sequence	Amplicon (bp)
Maco-A lef 7 for2	TACATTGATGCTGAAGCG	Maco-A lef 7 rev2	TCATGTTGTAATTGTCGTC	195
Maco-A orf 4 F1	GACGATGATGAAGAAAACC	Maco-A orf 4 R1	GGGTGAGTCGACTTGCGT	579
Maco-B orf 4 F2	CCAAAAATCATCTCGCCGAA	Maco-B orf 4 R1	TTGCTTTGTCATTTTCGACG	458/488

technology.

The sequence reads for each virus isolate were assembled using CLC-Genomics Workbench v8.5.1 for *de novo* assembly of reads into contig(s) [word size 64; bubble size 1000] and in most cases one or two large contigs were assembled. When more than one CLC contig was generated upon initial assembly, the SeqMan-Pro platform (DNASTAR-Lasergene v11) was used for the potential joining of contigs based on overlapping sequence homology. Where gaps occurred, coverage was low (<50x), or at a genome circularization/linearization position; PCR primers were designed to amplify the regions in questions and the amplicons were Sanger dideoxy sequenced to fill-in and confirm genome sequences. The statistics for the 454-sequence read number, average read length, assembled contig/genome size; as well as average read coverage and % of total reads that mapped to the assembled genome are listed in Table 2. The final genome assembly sequence was analyzed for ORF identifications and annotation using Gene Quest (DNASTAR-Lasergene v11). The ORFs were manually annotated by selecting identified ORFs on either strand that were at least 50 codons or had minimal overlap with adjacent ORFs. Some latitude was allowed for slightly shorter ORFs that were identified as having similarity to previously identified baculovirus ORFs based on predicted amino acid sequence identity. BLASTP was used to assess predicted aa identity to ORFs identified in previously sequenced MacoNPV-A, MacoNPV-B or other baculoviruses.

Whole genome sequence alignments were done using NCBI BLAST Suite "Align Two Nucleotide Sequences BLAST" tool in order to compare each Eurasian MbNPV/MabrNPV genome sequence to its respective MacoNPV-A or MacoNPV-B North American counterpart. The mean nucleotide % identity estimates were derived from summed number of identical nucleotides divided by total nucleotides aligned minus gaps for all aligned segments for each virus isolate genome. The progressiveMauve tool in MegAlign Pro (DNASTAR-Lasergene v13) was used for multiple global genome alignments. MegAlign Pro tool (DNASTAR-Lasergene v13) uses the progressiveMauve based algorithm of Darling *et al.*, 2010 which incorporates an alignment objective score known as "sum-of-pairs breakpoint score" and probabilistic alignment filtering method to remove erroneous alignments (Darling *et al.*, 2010). The program generates inferred unrooted phylogenetic trees based on the distance estimates from the alignment using a BioNJ algorithm (Gascuel, 1997).

2.6. Identity-gene parity plot analysis

Gene arrangements in baculoviruses have previously been compared using a GeneParityPlot approach (Hu *et al.*, 1998) in which the X-axis is used to represent the gene order of one virus, and the Y-axis to represent the gene order in a second virus. A modified Identity-GeneParityPlot approach that uses various colors to represent homology levels between individual genes of the two viruses was developed to provide a second level of information for the analysis of whole genomes not only comparing gene arrangement but also homology levels of individual genes (Li, L. *et al.*, 2002). The Identity-GeneParityPlot was used for whole genome comparison to show relative locations of individual ORFs of MacoNPV-96B or MacoNPV-90/2 versus the ORFs in newly sequenced genomes of Eurasian MabrNPV isolates based on the ORF identification and analysis results given in Tables 3 and 4. For each genome the *polyhedrin* gene was called ORF 1 with the remaining ORFs continuing from the 3′ end of the *polyhedrin* gene. The corresponding box for each gene pair was color coded according to the level of amino acid identity.

Phylogenetic analysis of putative amino acid sequences predicted from baculovirus ORF was undertaken via alignment of multiple

Table 2
Genome characteristics of alphabaculoviruses of *Mamestra*.

	Isolate	Locale	Total Genome Size (bp)	Total Reads	Coverage	# of ORFs	#bro genes	% GC	Reference / NCBI Accession #
MacoNPV-A Group	MabrNPV-Bu	Kostinbrod, Bulgaria	1,52,892	1,59,547	556	166	7	41.39	This study /OR184841
	MacoNPV-A 90/4	Lamont, AB	1,53,656	NA	NA	168	7	41.74	Li, L. et al., 2005 / AF539999.1
	MabrNPV-Uk	Kyvi, Ukraine	1,54,656	1,36,769	450	169	7	41.35	This study /OR184842
	MacoNPV-A AB260	La Crete, AB	1,54,861	68,833	264	169	8	41.42	This study / MK409385.1
	MacoNPV-A SK156	Davidson, SK	1,54,963	56,964	224	169	8	41.70	This study / MK252969.1
	MacoNPV-A 90/2	Wilkie, SK	1,55,060	NA	NA	169	8	41.70	Li, Q. et al., 2002 / NC_003529.1
	MacoNPV-A Ls*	Yakama WA, USA	1,57,635	59,922	130	170	8	41.76	This study / MN395659
MacoNPV-B Group	MabrNPV-Si	Novosibirsk, Russia	1,52,176	1,20,748	661	160	6	40.17	This study /OR184844
	MabrNPV-De	Heidelberg, Germany	1,52,272	87,348	410	160	6	40.17	This study /OR271605
	MabrNPV-K1	South Korea	1,52,710	NA	NA	159	6	39.89	Choi, et al., 2013 / NC_023681.1
	MabrNPV-Nl	Wageningen, Netherlands	1,52,711	1,47,114	465	160	6	39.90	This study /OR184843
	MacoNPV-B SK256	Davidson, SK	1,53,363	82,577	397	161	7	40.00	This study / MN337871
	MabrNPV-Cta	Tian City, China	1,53,890	NA	NA	162	6	40.09	/ KJ871680
	HearMNPV	Shanghi, China	1,54,196	NA	NA	162	6	40.07	Tang, et al., 2012
	MabrNPV-CHb1	Hebei, China	1,54,451	NA	NA	162	6	40.06	/ JX138237.2
	MacoNPV-B 96/2	Champion, AB	1,58,481	NA	NA	168	7	40.04	Li, L. et al., 2002 / NC_004117.1

* 454 vs 454 FLX Titatum Sequenceing.
MacoNPV-A Ls variant isolated from *Laconobia subjuncta*.

Table 3
Eurasian MacoNPV-A ORFs compared to MacoNPV-A 90/2.

MacoA-90/2 Number (name)	size (aa)	orf#	Mabr-UK size (aa)	% ID (range)	orf#	Mabr-Bu size (aa)	% ID (range)	Unique or Variable ORFs	AcMNPVHomologue
1 (polh)	246	1	246	100 % (246/246)	1	246	100 % (246/246)		ac8
2 (orf1629)	466	2	466	96 % (449/466)	2	466	96 % (449/466)		ac9
3 (pk1)	272	3	272	98 % (267/272)	3	272	97 % (265/272)		ac10
4 (hoar)	722	4	721	95 % (688/726)	4	722	94 % (686/727)		
5	202	5	198	96 % (194/202)	5	193	96 % (188/202)		
6 (pif-5)	373	6	373	97 % (362/372)	6	373	97 % (362/372)		ac148
7 (me53)	354	7	354	88 % (311/354)	7	354	92 % (324/354)		ac139
8	67	8	67	94 % (63/67)	8	67	93 % (62/67)		ac152
9 (fusion protein)	680	9	681	97 % (661/681)	9	681	97 % (661/681)		ac23
10	319	10	319	98 % (312/319)	10	319	98 % (312/319)		
11 (gp16)	95	11	95	100 % (95/95)	11	95	100 % (95/95)		ac130
12 (p24)	228	12	231	98 % (227/231)	12	231	98 % (227/231)		ac129
13	102	13	101	99 % (101/102)	13	101	99 % (101/102)		
14 (lef-2)	211	14	210	97 % (205/211)	14	210	98 % (206/211)		ac6
15 (xe-1)	138	15	124	98 % (121/124)	15	124	97 % (120/124)		
hr1									
16 (lef-7)	172	16	172	96 % (165/172)	16	172	96 % (165/172)		ac79
17	92	17	92	99 % (91/92)	17	92	100 % (92/92)		
18	199	18	199	98 % (196/199)	18	199	98 % (196/199)		
19	236	19	233	83 % (181/217)	19	236	84 % (182/217)		
20	175								
21 (bro-a)	161								ac2
		20	216	62 % (129/208)				ORF66 [Xec-n GV]	
22 (chit)	562	21	562	99 % (560/562)	20	562	99 % (561/562)		ac126
23	85	22	76	95 % (70/74)					
24 (bro-b)	372	23	480	80 % (266/331)	21	480	81 % (267/331)		ac2
25	141	24	139	91 % (125/138)	22	139	95 % (131/138)		
26	270	25	295	77 % (207/269)	23	269	77 % (208/269)		
		26	198		24	198			
27	211	27	211	99 % (210/211)	25	211	99 % (210/211)		
28	231	28	231	94 % (216/231)	26	231	94 % (218/231)		
29	110	29	85	92 % (58/63)	27	83	92 % (58/63)		
30	214	30	213	89 % (190/214)	28	213	90 % (192/214)		
		31	91	71 % (64/90)				bro like (bro-c)	
		32	119	40 % (30/75)				bro like (bro-c)	
31 (bro-c)	486	33	477	79 % (385/486)	29	474	83 % (401/486)		ac2
32 (he65)	558	34	559	96 % (536/558)	30	559	96 % (536/558)		ac105
33 (cath)	337	35	337	99 % (335/337)	31	337	99 % (335/337)		ac127
34	117	36	118	91 % (107/117)	32	118	91 % (106/117)		
35 (lef-1)	215	37	215	99 % (214/215)	33	215	99 % (212/215)		ac14
36	350	38	350	98 % (342/350)	34	350	98 % (343/350)		ac13
37 (gp37)	262	39	262	98 % (257/262)	35	262	98 % (258/262)		ac64
38 (ptp-2)	179	40	179	96 % (172/179)	36	179	98 % (175/179)		
39 (egt)	516	41	516	98 % (506/516)	37	516	98 % (506/516)		ac15
40	177	42	198	100 % (177/177)	38	198	100 % (177/177)		
41	213	43	213	98 % (209/213)	39	213	98 % (209/213)		ac17
42	848	44	848	98 % (827/848)	40	848	98 % (827/848)		
43	150	45	150	92 % (138/150)	41	150	93 % (139/150)		ac145
44	173	46	235	96 % (213/236)	42	235	95 % (169/177)		
45 (pkip)	168	47	169	98 % (165/169)	43	169	97 % (159/164)		ac24
46	113	48	113	100 % (113/113)	44	113	100 % (113/113)		
47 (arif-1)	290	49	290	98 % (284/290)	45	290	98 % (284/290)		ac22
48 (pif-2)	419	50	419	99 % (414/419)	46	419	99 % (414/419)		ac119
49 (pif-1)	529	51	529	99 % (527/529)	47	529	99 % (524/529)		
50	81	52	81	99 % (80/81)	48	81	99 % (80/81)		
51 (fgf)	373	53	372	94 % (350/373)	49	372	94 % (349/373)		ac32
52	64	54	64	98 % (62/63)	50	64	98 % (62/63)		
53	238	55	238	97 % (231/238)	51	238	97 % (231/238)		
54 (alk-exo)	395	56	395	92 % (362/395)	52	395	92 % (362/395)		ac133
55	110	57	110	97 % (107/108)	53	110	98 % (108/110)		ac19
56	389	58	388	99 % (385/388)	54	388	99 % (385/388)		ac18
57	135	59	135	99 % (133/135)	55	135	99 % (134/135)		
58 (rr2b)	313	60	313	99 % (309/311)	56	313	99 % (310/313)		
59	338								
60 (calyx)	321	61	321	100 % (321/321)	57	321	100 % (321/321)		ac131
61	225								
62	113	62	97	83 % (81/98)	58	97	83 % (81/98)		ac117
63	120	63	120	92 % (110/120)	59	120	93 % (111/120)		
64	221	64	221	88 % (196/221)	60	222	88 % (187/209)		
65	204	65	204	98 % (199/204)	61	128	95 % (122/128)	3′ truncated	
66 (sod)	151	66	151	99 % (150/151)	62	151	99 % (149/151)		ac31
67	118	67	161	97 % (154/161)	63	161	96 % (151/161)		
68 (pif-3)	203	68	203	100 % (203/203)	64	203	99 % (202/203)		ac115

(*continued on next page*)

Table 3 (*continued*)

MacoA-90/2 Number (name)	size (aa)	orf#	Mabr-UK size (aa)	% ID (range)	orf#	Mabr-Bu size (aa)	% ID (range)	Unique or Variable ORFs	AcMNPVHomologue
69	158	69	158	99 % (156/158)	65	158	98 % (155/158)		
70 (parg)	483	70	483	97 % (473/483)	66	483	97 % (469/483)		
71	216	71	216	99 % (213/216)	67	216	99 % (213/216)		ac106/107
72 (pnk)	359	72	359	98 % (354/359)	68	359	99 % (356/359)		ac33
hr2									
73	157	73	157	95 % (149/157)	69	157	96 % (150/157)		ac4
74 (dUTPase)	142	74	142	97 % (138/142)	70	142	96 % (137/142)		
75 (bro-d)	329	75	326	94 % (308/326)	71	329	93 % (305/329)		ac2
76 (p13)	276	76	278	99 % (273/276)	72	278	99 % (273/276)		
77 (xe-2)	174	77	174	99 % (173/174)	73	174	99 % (172/174)		
78 (odv-e66a)	672	78	672	99 % (669/672)	74	672	99 % (668/672)		ac46
79	103	79	103	97 % (100/103)	75	103	97 % (100/103)		ac108
80	356	80	356	99 % (355/356)	76	356	99 % (355/356)		ac109
81	59	81	59	100 % (59/59)	77	59	100 % (59/59)		ac110
82 (vp80)	547	82	547	96 % (527/547)	78	547	96 % (527/547)		ac104
83 (p45)	377	83	377	99 % (375/377)	79	377	99 % (375/377)		ac103
84 (p12)	102	84	102	98 % (100/102)	80	102	98 % (100/102)		ac102
85 (bv/odv-c42)	365	85	365	99 % (361/365)	81	365	99 % (363/365)		ac101
86 (p6.9)	76	86	76	100 % (76/76)	82	76	100 % (76/76)		ac100
87 (lef-5)	273	87	273	99 % (272/273)	83	273	99 % (270/273)		ac99
88 (38 K)	301	88	301	99 % (299/301)	84	301	99 % (299/301)		ac98
89 (vef)	847	89	847	96 % (810/847)	85	847	96 % (819/847)		
90 (bro-e)	360	90	360	99 % (356/360)	86	360	99 % (359/360)		ac2
91	142	91	142	99 % (141/142)	87	142	99 % (141/142)		
92 (pif-4)	172	92	172	100 % (172/172)	88	172	100 % (172/172)		ac96
93 (hel-1)	1212	93	1211	99 % (1208/1212)	89	1213	99 % (1209/1212)		ac95
94 (odv-e25)	216	94	216	99 % (215/216)	90	216	99 % (214/216)		ac94
95	161	95	161	100 % (161/161)	91	161	100 % (161/161)		ac93
96 (sox)	252	96	252	99 % (249/252)	92	252	98 % (248/252)		ac92
97	168	97	168	98 % (165/168)	93	168	99 % (166/168)		
98 (lef-4)	454	98	454	98 % (446/454)	94	454	98 % (449/454)		ac90
99 (vp-39)	325	99	325	95 % (309/325)	95	325	95 % (309/325)		ac89
100 (cg30)	279	100	277	96 % (269/279)	96	277	97 % (260/279)		ac88
101 (vp91)	809	101	816	96 % (796/809)	97	816	96 % (782/809)		ac83
102 (tlp)	195	102	193	98 % (191/195)	98	193	99 % (192/193)		ac82
103	238	103	238	99 % (237/238)	99	238	99 % (237/238)		ac81
104 (gp41)	333	104	333	99 % (329/333)	100	333	99 % (329/333)		ac80
105	114	105	114	99 % (113/114)	101	114	99 % (113/114)		ac78
106 (vlf-1)	380	106	379	98 % (374/380)	102	380	99 % (377/380)		ac77
107 (ctl)	50	107	92	96 % (48/50)	103	92	96 % (48/50)	5′ extension	ac3
108	364	108	368	89 % (328/368)	104	368	90 % (329/368)		
109 (p26a)	244	109	244	99 % (242/244)	105	244	99 % (242/244)		ac136
110 (iap-2)	252	110	252	98 % (247/252)	106	252	98 % (247/252)		ac71
111 (Mtase)	275	111	275	98 % (270/275)	107	275	98 % (270/275)		ac69
112 (pif-6)	121	112	121	99 % (120/121)	108	121	99 % (120/121)		ac68
113 (lef-3)	385	113	385	99 % (381/385)	109	385	98 % (378/385)		ac67
114	740	114	749	89 % (664/749)	110	750	89 % (664/749)		ac66
115 (DNApol)	998	115	998	98 % (974/998)	111	998	98 % (974/998)		ac65
116	129	116	129	98 % (126/129)	112	129	98 % (126/129)		ac75
117	85	117	85	100 % (85/85)	113	85	98 % (84/85)		ac76
118	249	118	249	98 % (244/249)	114	249	96 % (241/249)		ac150
119	181	119	181	99 % (179/181)	115	181	99 % (180/181)		
120	156	120	156	99 % (155/156)	116	156	99 % (155/156)		
121	215	121	215	95 % (204/215)	117	215	96 % (208/215)		
122 (bro-f)	357	122	357	96 % (343/357)	118	328	86 % (290/337)		ac2
123 (bro-g)	235	123	141	63 % (74/117)	119	140	55 % (79/143)	3′ truncated	ac2
124 (lef-9)	505	124	505	99 % (500/505)	120	505	99 % (500/505)		ac62
125 (fp-25 k)	195	125	195	100 % (195/195)	121	195	100 % (195/195)		ac61
126 (p94)	819	126	834	90 % (734/819)	122	834	91 % (740/819)		ac134
127 (bro-h)	179	127	179	98 % (175/179)	123	179	99 % (178/179)		ac2
128 (ChaB-like)	90	128	90	98 % (88/90)	124	90	98 % (88/90)		ac60
129 (ChaB-like)	166	129	164	94 % (156/166)	125	164	99 % (164/166)		ac58/59
130	159	130	159	99 % (158/159)	126	159	99 % (158/159)		ac57
131	89	131	89	98 % (87/89)	127	89	98 % (87/89)		ac56
132	71	132	72	99 % (71/72)	128	72	99 % (71/72)		ac55
133 (vp1054)	336	133	336	99 % (333/336)	129	336	99 % (332/336)		ac54
134 (lef-10)	75	134	75	100 % (75/75)	130	75	100 % (75/75)		ac53a
135	75	135	75	100 % (75/75)	131	75	100 % (75/75)		
136	311	136	311	96 % (300/311)	132	311	96 % (300/311)		
137	157	137	157	99 % (156/157)	133	157	99 % (156/157)		ac53
138	166	138	166	99 % (164/166)	134	166	99 % (164/166)		ac52
hr3									
139 (iap-3)	276	139	284	89 % (255/285)	135	284	89 % (255/285)		ac27
140 (bjdp)	388	140	390	97 % (379/390)	136	388	97 % (377/388)		ac51
141 (lef-8)	878	141	878	99 % (871/878)	137	878	99 % (875/878)		ac50

(*continued on next page*)

Table 3 (*continued*)

MacoA-90/2 Number (name)	size (aa)	orf#	Mabr-UK size (aa)	% ID (range)	orf#	Mabr-Bu size (aa)	% ID (range)	Unique or Variable ORFs	AcMNPVHomologue
142	152	142	152	96 % (146/152)	138	152	97 % (148/152)		
143	66	143	66	98 % (65/66)	139	66	98 % (65/66)		ac43
144 (odv-e66b)	666	144	670	98 % (654/670)	140	670	98 % (654/670)		ac46
145 (p47)	397	145	397	98 % (388/397)	141	397	98 % (389/397)		ac40
146	349	146	349	88 % (309/350)	142	349	89 % (313/349)		
hr4									
147	193	147	194	98 % (190/193)	143	128	99 % (127/128)	internal deletion	
148 (nudix)	230	148	230	99 % (227/230)	144	230	99 % (228/230)		ac38
149 (lef-11)	120	149	120	97 % (116/120)	145	120	98 % (117/120)		ac37
150 (39k)	287	150	287	98 % (282/287)	146	287	97 % (280/287)		ac36
151	65	151	65	97 % (63/65)	147	65	97 % (63/65)		
152 (v-ubi)	100	152	100	97 % (97/100)	148	100	97 % (97/100)		ac35
153	181	153	181	99 % (180/181)	149	181	99 % (180/181)		ac34
154	119	154	119	99 % (116/117)	150	119	100 % (119/119)		ac26
155 (dbp)	328	155	328	99 % (327/328)	151	328	99 % (327/328)		ac25
156 (lef-6)	143	156	143	100 % (143/143)	152	143	100 % (143/143)		ac28
157	81	157	81	100 % (81/81)	153	81	100 % (81/81)		ac29
158 (p26b)	267	158	266	100 % (266/266)	154	266	100 % (266/266)		ac136
159 (p10)	85	159	85	100 % (85/85)	155	85	98 % (84/85)		ac137
160 (pif-0)	657	160	657	99 % (655/657)	156	657	100 % (657/657)		ac138
161	85	161	85	100 % (85/85)	157	85	100 % (85/85)		
162 (ie-1)	606	162	603	98 % (594/607)	158	603	98 % (593/607)		ac147
163	191	163	191	99 % (190/191)	159	191	99 % (190/191)		ac146
164	92	164	92	99 % (91/92)	160	92	99 % (91/92)		ac145
165 (odv-ec27)	278	165	278	99 % (277/278)	161	278	100 % (278/278)		ac144
166 (odv-e18)	83	166	83	98 % (81/83)	162	83	98 % (81/83)		ac143
167 (p49)	461	167	461	99 % (459/461)	163	461	99 % (459/461)		ac142
168 (ie0/exon0)	234	168	234	99 % (232/234)	164	232	99 % (232/234)		ac141
169 (rr1)	761	169	761	98 % (748/761)	165	761	98 % (749/761)		

proteins. Maximum likelihood phylogenetic trees were generated using the Neighbour Joining algorithm with the WAG amino acid substation model and 500 rounds of boot strap analysis in the CLC Genomics Workbench platform.

2.7. Bioassays

To compare the infectivity and virulence of the various virus isolates a 2-dose ($1.0x10^5$ and $1.0x10^7$ OB/ml) bioassay was conducted using a droplet feeding exposure assay with 2nd instar *M. configurata* or *T. ni* larvae. The larvae were starved overnight prior to the droplet feeding exposure with the virus applied in 5 μL droplets containing virus OB dilutions in 1 % sucrose, 5 % blue food coloring solution. Only larvae feeding on the virus suspension within 15 min were included in the assay. The larvae were reared individually in 12 well tissue culture plates (Costar, Corning, NY) with appropriate artificial diet sources. Three replicate assays using different cohorts of insects were conducted with 24 larvae per dose per experiment. Final virus-related mortality was enumerated at day 10 post inoculation (pi). Mortality data was analyzed using a one-way ANOVA (STATISTIXv10, FL).

3. Results

3.1. Multiplex PCR & REN analysis

The DNA preparations from the Eurasian 'MabrNPV' isolates were characterized using the MacoNPV-A and MacoNPV-B species-specific multiplex PCR assay based on differences in *hoar* gene sequences and the presence/absence of the *lef-7* gene. The MabrNPV-Uk and MabrNPV-Bu isolate DNAs produced the same sized 579 bp hoar amplicons as the MacoNPV-A 90/2 North American isolate as well as a 195 bp *lef-7* product (Fig. 1). In contrast only smaller *hoar* amplicons were produced for the MacoNPV-B like isolates MacoNPV-B 96/2, MabrNPV-Si and -Nl (Fig. 1). The *hoar* amplicon sizes displayed slight differences in migration distances reflecting the differences in the *hoar* gene sequences in the different MacoNPV-B like isolates. Interestingly, the MabrNPV-Yu isolate produced all three amplicons indicating it was a mixture of both virus species, but the band intensity suggested that the MacoNPV-B type predominated. These results indicate that both MacoNPV-A and MacoNPV-B type viruses exist and circulate in *M. brassicae* Eurasian populations.

The multiplex PCR results were confirmed by *Hin*dIII restriction digests of the virus DNAs. The *Hin*dIII fragment profile for each of the Eurasian isolates was identical to that previously reported by van Oers and Vlak (2007) (data not shown). As previously demonstrated the *Hin*dIII fragment profile for MabrNPV-Yu contained many submolar bands confirming that it contained a mixture of genotypes.

3.2. Genome comparisons

The complete genome sequence of the five Eurasian alphabaculovirus isolates, MabrNPV-UK, -Bu, -Nl, -DE and -Si from *M. brassicae* populations as well as three North American isolates MacoNPV-A SK156, -AB260, -Ls and MacoNPV-B SK256 were assembled and annotated. These sequences were compared to each other as well as the previously published genomes of other MacoNPV/MabrNPV-like viruses, including; MacoNPV-A 90/2, MacoNPV-B 96/2, MabrNPV-K1, MabrNPV-CHb1 and HearMNPV (Table 2). The size of each genome, number of *bro*-like genes and total number of *orf*s are summarized in Table 2. The MacoNPV-A like genomes ranged in size from 152,892 bp (MabrNPV-Bu) with 166 identified ORFs to 157,635 bp (MacoNPV-Ls) with 170 identified ORFs. The MacoNPV-B like genomes ranged in size from 152,176 bp (MabrNPV-Si) with 160 identified ORFs to 158,481 bp (MacoNPV-B 96/2) with 168 identified ORFs. The largest contribution to the difference between MacoNPV-B 96/2 and the other MacoNPV-B like genomes is a ~ 5,400 bp insertion between *orf53* and the *bro-b* gene of a sequence homologous to a region of Xestia c-nigrum granulovirus (XecnGV). We also sequenced an additional MacoNPV-B like isolate, MacoNPV-B SK256, from North America for comparison and significantly the genome lacked the large XecnGV insert found in MacoNPV-B 96/2. The genome size of MacoNPV-B SK256 was therefore much more similar to that of the Eurasian MacoNPV-B type isolates. The genomes of the MabrNPV isolates -De and -Si were nearly identical (>99.9 % nucleotide ID) with minor differences occurring in *hr1*and an

Table4
Eurasian MacoNPV-B strain ORFs compared with MacoNPV-B 96/2.

MacoB-Si size (aa)	% ID (range)	Closest identity of MacoNPV-B-NL or -Si ORFs if not to the archytype MacoNNPV-B-96/2 genome (Bold text- MacoB-Si Only)	AcMNPV ORF
246	100 % (246/246)		ac8
506	99 % (506/512)		ac9
272	100 % (272/272)		ac10
740	99 % (740/748)		
188	97 % (185/190)		
373	100 % (373/373)		ac148
354	100 % (354/354)		ac139
678	99 % (675/678)		ac23
316	99 % (316/319)		
95	100 % (95/95)		ac130
229	100 % (229/229)		ac129
103	99 % (103/104)		
215	99 % (213/215)		ac6
127	98 % (124/127)		
98	100 % (98/98)		ac79
199	99 % (197/199)		
236		Maco-A 19 179/218(82 %)	
562	99 % (559/562)		ac126
488	75 % (269/359)		ac2
141	99 % (140/141)		
269	98 % (264/269)		
211	99 % (208/211)		
104	99 % (103/104)		
211	98 % (207/211)		
455	99 % (449/455)		
584	99 % (579/584)		ac105
341	100 % (341/341)		ac127
115	100 % (115/115)		
215	99 % (213/215)		ac14
349	100 % (349/349)		ac13
262	99 % (260/262)		ac64
179	98 % (176/179)		
528	99 % (513/516)		ac15
178	99 % (177/178)		
216	99 % (211/213)		ac17
851	99 % (849/851)		
146	99 % (145/146)		ac145
175	98 % (172/175)		
169	100 % (169/169)		ac24
137	96 % (101/105)	3′ extension	
290	99 % (287/290)		ac21
419	99 % (417/419)		ac22
529	99 % (526/529)		ac119
81	100 % (81/81)		
363	95 % (345/364)		ac32
238	89 % (226/238)	5′ truncation	
395	90 % (354/395)	92 % ID to MacoA-90/2 alk-exo	ac133
110	96 % (106/110)	98 % ID to MacoA-90/2 orf 49	ac19
388	94 % (362/388)	99 % ID to MacoA-90/2 orf 50	ac18
135	94 % (124/132)	99 % ID to MacoA-90/2 orf 51	
313	94 % (294/313)	99 % ID to MacoA-90/2 rr2b	
321	88 % (285/324)	99 % ID to MacoA-90/2 calyx	ac2 ac133
97	98 % (95/97)	5 ’truncation	ac117
120	93 % (112/120)		
224	88 % (194/219)	maco-A 64	
204	87 % (178/204)	97 % ID to MacoA-90/2 orf 64	
151	99 % (149/151)		ac31
161	87 % (105/121)	5′ extension	
203	93 % (188/203)	100 % ID to MacoA-90/2 pif-3	ac115
158	88 % (136/155)	98 % ID to MacoA-90/2 orf 68	
483	88 % (427/484)	97 % ID to MacoA-90/2 orf 69	
216	95 % (206/216)	99 % ID to MacoA-90/2 orf 70	ac106/107
359	95 % (341/359)	99 % ID to MacoA-90/2 pnk	ac33
157	89 % (141/158)	95 % ID to MacoA-90/2 orf 72	ac4
142	97 % (138/142)		
326	94 % (308/326)		ac2

(*continued on next page*)

Table4 (*continued*)

MacoB-Si size (aa)	% ID (range)	Closest identity of MacoNPV-B-NL or -Si ORFs if not to the archytype MacoNNPV-B-96/2 genome (Bold text- MacoB-Si Only)	AcMNPV ORF
278	96 % (266/278)	99 % ID to MacoA-90/2 p13	
174	91 % (158/174)	99 % ID to MacoA-90/2xe-2	
672	98 % (637/653)	99 % ID to MacoA-90/2 odv-e66	ac46
103	94 % (97/103)		ac108
356	95 % (339/356)	99 % ID to MacoA-90/2 orf 79	ac109
59	98 % (58/59)	100 % ID to MacoA-90/2 orf 80	ac110
547	83 % (440/548)	97 % ID to MacoA-90/2 vp80	ac104
374	98 % (376/374)	99 % ID to MacoA-90/2 p45	ac103
102	89 % (91/102)	98 % ID to MacoA-90/2 p12	ac102
365	93 % (336/364)	99 % ID to MacoA-90/2 bv/odv-c42	ac101
76	78 % (60/76)	100 % ID to MacoA-90/2 p6.9	ac100
273	99 % (270/273)		ac99
301	96 % (288/300)	99 % ID to MacoA-90/2 38 K	ac98
848	96 % (814/848)		
356	99 % (351/356)		ac2
142	100 % (142/142)		
172	99 % (171/172)		ac96
1209	99 % (1206/1209)		ac95
216	100 % (216/216)		ac94
161	100 % (161/161)		ac93
252	99 % (250/252)		ac92
171	98 % (171/174)		
454	99 % (453/454)		ac90
328	99 % (327/328)		ac89
274	99 % (270/274)		ac88
812	99 % (806/812)		ac83
195	98 % (192/195)		ac82
240	99 % (239/240)		ac81
333	100 % (333/333)		ac80
151	100 % (151/151)		ac78
380	100 % (380/380)		ac77
50	96 % (48/50)		ac3
364	98 % (357/364)		
242	99 % (242/244)		ac136
248	99 % (247/248)		ac71
275	99 % (271/275)		ac69

Table4 (*continued*)

MacoB-Si size (aa)	% ID (range)	Closest identity of MacoNPV-B-NL or -Si ORFs if not to the archytype MacoNNPV-B-96/2 genome (Bold text- MacoB-Si Only)	AcMNPV ORF
121	100 % (121/121)		ac68
393	99 % (390/393)		ac67
751	98 % (734/752)		ac66
1000	100 % (1000/1000)		ac65
129	100 % (129/129)		ac75
85	100 % (85/85)		ac76
246	98 % (240/246)		ac150
181	99 % (179/181)		
153	99 % (152/153)		
215	97 % (209/215)		
345	94 % (327/349)		ac2
229	99 % (226/229)		ac2
497	100 % (497/497)		ac62
195	100 % (195/195)		ac61
834	99 % (830/834)		ac134
162	99 % (161/162)		ac2
97	100 % (93/93)		ac60
162	97 % (162/167)		ac59
159	100 % (159/159)		ac57
89	100 % (89/89)		ac56
69	100 % (69/69)		ac55
336	99 % (334/336)		ac54
75	99 % (74/75)		ac53a
75	99 % (74/75)		
335	92 % (310/335)		
157	100 % (157/157)		ac53
167	100 % (167/167)		ac52
285	99 % (284/285)		ac27
384	99 % (379/384)		ac51
878	99 % (877/878)		ac50
154	98 % (151/154)		
69	100 % (65/65)		ac43
671	95 % (640/671)		ac46
397	97 % (386/397)		ac40
349	90 % (313/349)		

(*continued on next page*)

Table4 (*continued*)

MacoB-Si size (aa)	% ID (range)	Closest identity of MacoNPV-B-NL or -Si ORFs if not to the archytype MacoNNPV-B-96/2 genome (Bold text- MacoB-Si Only)	AcMNPV ORF
194	82 % (158/193)	98 % ID to MacoA-90/2 orf 146	
234	99 % (233/234)		ac38
124	99 % (123/124)		ac37
284	99 % (282/284)		ac36
65	100 % (65/65)		
100	100 % (100/100)		ac35
181	98 % (178/181)		ac34
118	99 % (117/118)		ac26
326	99 % (325/326)		ac25
141	99 % (139/141)		ac28
81	100 % (81/81)		ac29
266	98 % (262/266)		ac136
83	100 % (83/83)		ac137
657	99 % (654/657)		ac138
83	100 % (83/83)		
604	99 % (599/604)		ac147
191	99 % (190/191)		ac146
92	100 % (92/92)		ac145
278	100 % (278/278)		ac144
85	100 % (85/85)		ac143
461	99 % (460/461)		ac142
234	100 % (234/234)		ac141
761	99 % (755/761)		

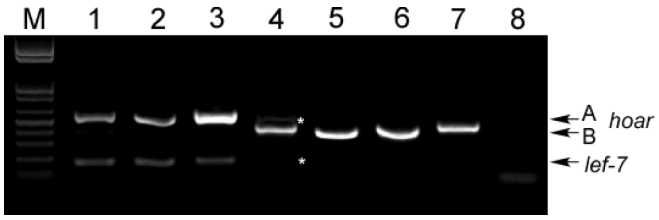

Fig. 1. Multiplex PCR assay for identification of MacoNPV-A and MacoNPV-B isolates. The DNA templates by lane include; 1) MabrNPV-Uk, 2) MabrNPV-Bu, 3) MacoNPV-A 90/2, 4) MabrNPV-Yu, 5) MacoNPV-B 96/2, 6) MabrNPV-Si, 7) MabrNPV-Nl, 8) no template control. The amplicon identification listed in the right margin refers to the MacoNPV-A *hoar* (579 bp), MacoNPV-B *hoar* (458–488 bp) and MacoNPV-A specific lef-7 (195 bp) amplicons.

8 bp region upstream of the *hoar* gene. All the putative amino acid sequences for the homologous ORFs were identical for the two virus isolates, thus only MabrNPV-Si was included in further comparative analysis. Tables 3 and 4 list the ORFs for the Eurasian isolates MabrNPV-Bu and MabrNPV-Uk relative to MacoNPV-A 90/2 and MabrNPV-Nl and MabrNPV-Si relative to MacoNPV-B 96/2, respectively. The percentage identity of the aa sequence relative to the MacoNPV-A 90/2 or MacoNPV-B 96/2 isolate homologues are provided.

The whole genome sequences confirmed that two of the Eurasian isolates, MabrNPV-Bu and –Uk, were isolates of MacoNPV-A. This assessment was based on general gene content, the presence of a *lef-7* homologue (Table 3), a higher GC ratio (41.4 %), and the high nucleotide identity relative to MacoNPV-A 90/2 across the length of the genome (96.2 and 95.6 % for MabrNPV-Bu and -Uk, respectively). MabrNPV-De, -Si, and -Nl isolates contained a *helicase-2* orthologue but lacked a *lef-7* orthologue, had lower GC ratios (39.9–40.2 %) and had very high nucleotide identity to MacoNPV-B 96/2 across the length of the genome (96.6, 96.6 and 98.5 %, respectively). Not surprisingly, the *hr* regions had lower identity (typically ~ 85 %) and varied in the number of repeats within the *hr*s for each of the isolate genomes.

3.3. Phylogenetic Analysis of lef-8, lef-9 and polh

For phylogenetic analyses, DNA sequences within the coding regions of three highly conserved genes, the *late expression factor 8* (*lef-8*), *late expression factor 9* (*lef-9*) and *polyhedrin* (*polh*) generated using degenerative primers, were used as recommended by Jehle et al. (2006). Supplemental Table 1 shows the Kimura two-parameter (K-2-P) distances for *lef-8, lef-9, polh* and the *lef-8/ lef-9/polh* concatenated sequence from the North American and Eurasian *Mamestra* baculovirus isolates. In almost all cases the K-2-P distances for MacoNPV-A 90/2, MacoNPV-A 90/4, MabrNPV-Uk and MabrNPV-Bu were 0.015 or lower indicating that they belonged to the same *Alphabaculovirus* species. The only exception was for the *lef-8* sequence for which MabrNPV-Bu and MabrNPV-Uk gave of values of 0.020 and 0.022, respectively. This same group of MacoNPV-A like viruses produced K-2-P distance values ranging from 0.06 to 0.092 versus the MacoNPV-B like viruses, substantially greater than 0.05 value cut off that indicates a separate *Alphabaculovirus* species. Similarly in all cases the K-2-P distances for MacoNPV-B 96/2, MabrNPV-Si, and MabrNPV-Nl were 0.004 or lower indicating that they belonged to the same *Alphabaculovirus* species.

3.4. Multiple genome alignments

We used a progressiveMauve based algorithm (Darling *et al.*, 2010) in the MegAlign Pro tool (DNASTAR-Lasergene v13) to simultaneously align the complete genome sequences of up to 12 isolates at one time. When we compared the complete genome sequences of the North American and Eurasian alphabaculoviruses sequenced for this study, the program aligned multiple genome sequences as single blocks with identified gaps (Fig. 2A). An inferred unrooted phylogenetic tree was generated from the alignment using a BioNJ algorithm. The resulting tree separated two clusters of related taxonomic units, one containing MacoNPV-A like isolates and the second containing MacoNPV-B like isolates (Fig. 2B). Each cluster contained isolates from both North America and Eurasia. Within the MacoNPV-A group the Eurasian isolates, MabrNPV-Uk and MabrNPV-Bu, always clustered together on a separate branch and among the North American isolates the MacoNPV-A SK156 isolate was the most distinct. Within the MacoNPV-B cluster, the two North American isolates included in the analysis, MacoNPV-B 96/2 and MacoNPV-B SK256, formed a branch with MabrNPV-Nl and the MabrNPV-Si isolate proved to be somewhat distinct. Inclusion of the Korean (MabrNPV-K1) and Chinese (MabrNPV-CHB1) MacoNPV-B isolates in the phylogenetic analysis produced similar results. The exception was that the MabrNPV-Nl grouped together with MabrNPV-K1 and MabrNPV-CHB1 on a separate branch from the North American MacoNPV-B isolates (Fig. 2B). The MabrNPV-Si isolate proved to be an even more distinct strain falling between the MacoNPV-A and MacoNPV-B like clusters. The exact elements driving the divergence of

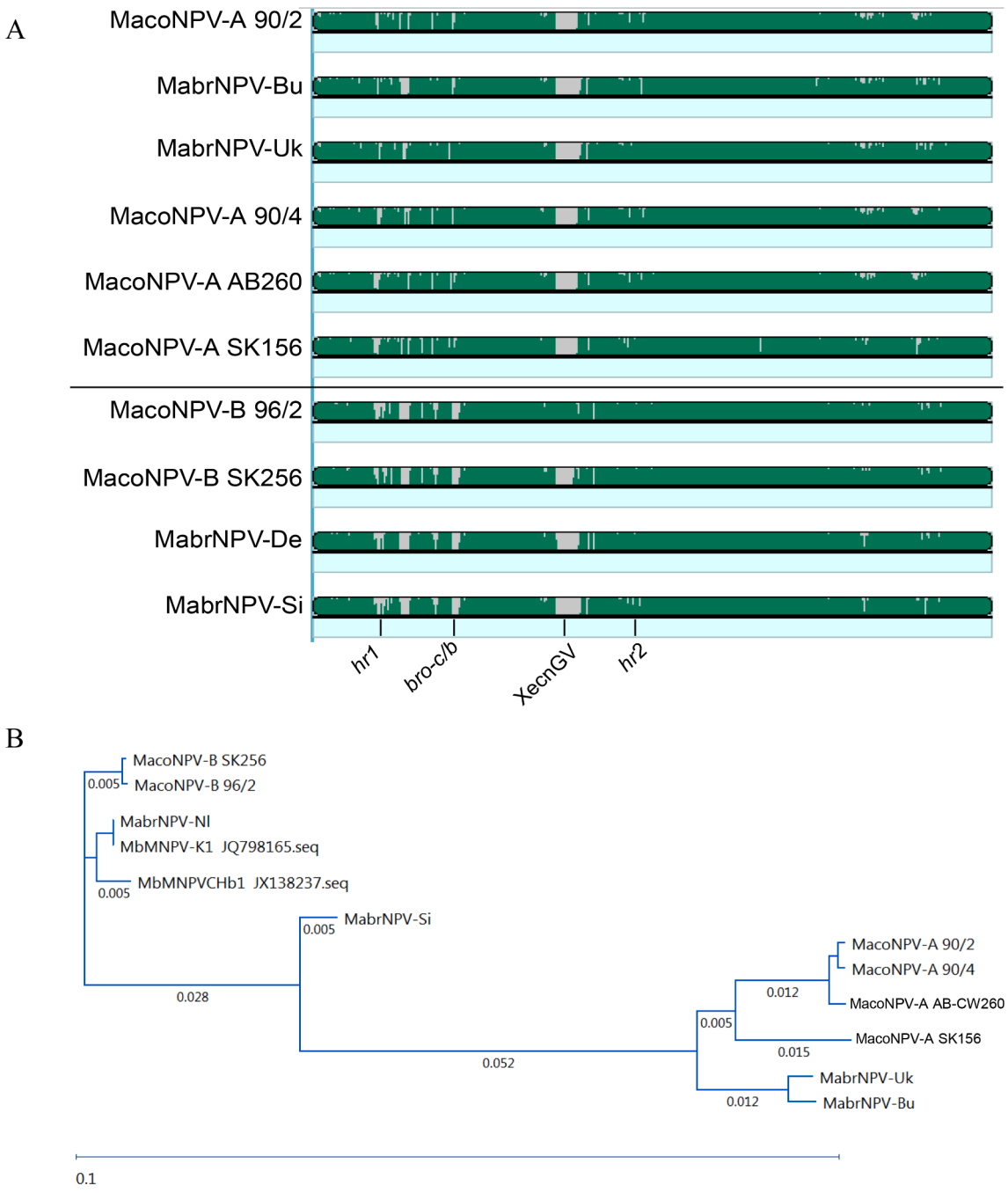

Fig. 2. Multiple genome alignment of North American and Eurasian MacoNPV-A and –B like isolates sequenced in this study using Progressive MAUVE and Inferred Phylogenetic Tree estimated using uncorrected pairwise distance metrics and BioNJ algorithm from the multiple sequence alignment. Panel A displays the alignment graphic with significant differences or gaps shaded in grey, several genomic landmarks are displayed on the bottom of the panel. Panel B shows the inferred phylogenetic tree with branch distances displayed.

the MabrNPV-Si isolate is not clear, but it may reflect the lower identity of the coding sequence of genes from ORF 46 through 85 compared to MacoNPV-B 96/2 and the other MacoNPV-B like genomes such as MabrNPV-Nl (Fig. 3D and Table 4). Indeed, many of the MabrNPV-Si ORFs from ORF46-85 had higher identity to MacoNPV-90/2 than to MacoNPV-B 96/2.

The general arrangement of *hr* regions and *bro* genes were similar for most of the MacoNPV-A viruses except for MacoNPV-A 90/2 which was the only isolate to contain a MacoNPV-A *bro-a* homologue (Fig. 4). Similarly, the MacoNPV-B type virus isolates had similar arrangements of *hr* regions and *bro* genes except that the North American isolates contained the MacoNPV-B *bro-b* homologue which was absent in all the Eurasian MacoNPV-B isolates.

3.5. ORF analysis -MacoNPV-A isolates

An Identity-Gene Parity Plot analysis was conducted for each of the Eurasian isolates in comparison to the relevant North American MacoNPV species genome (Fig. 3A-D). The Identity-Gene Parity Plot analysis for MabrNPV-Uk versus MacoNPV-A 90/2 showed the gene order on the two genomes are co-linear except that there are 4 ORFs unique to MacoNPV-A 90/2 (ORFs 20, BRO-A, 59 and 61) and 4 ORFs are unique to MabrNPV-Uk (ORFS Xecn66-like, 26 and 31 and 32 [two *bro*-like genes]) (Fig. 3A). MabrNPV-Uk ORF 31 and 32 are smaller than

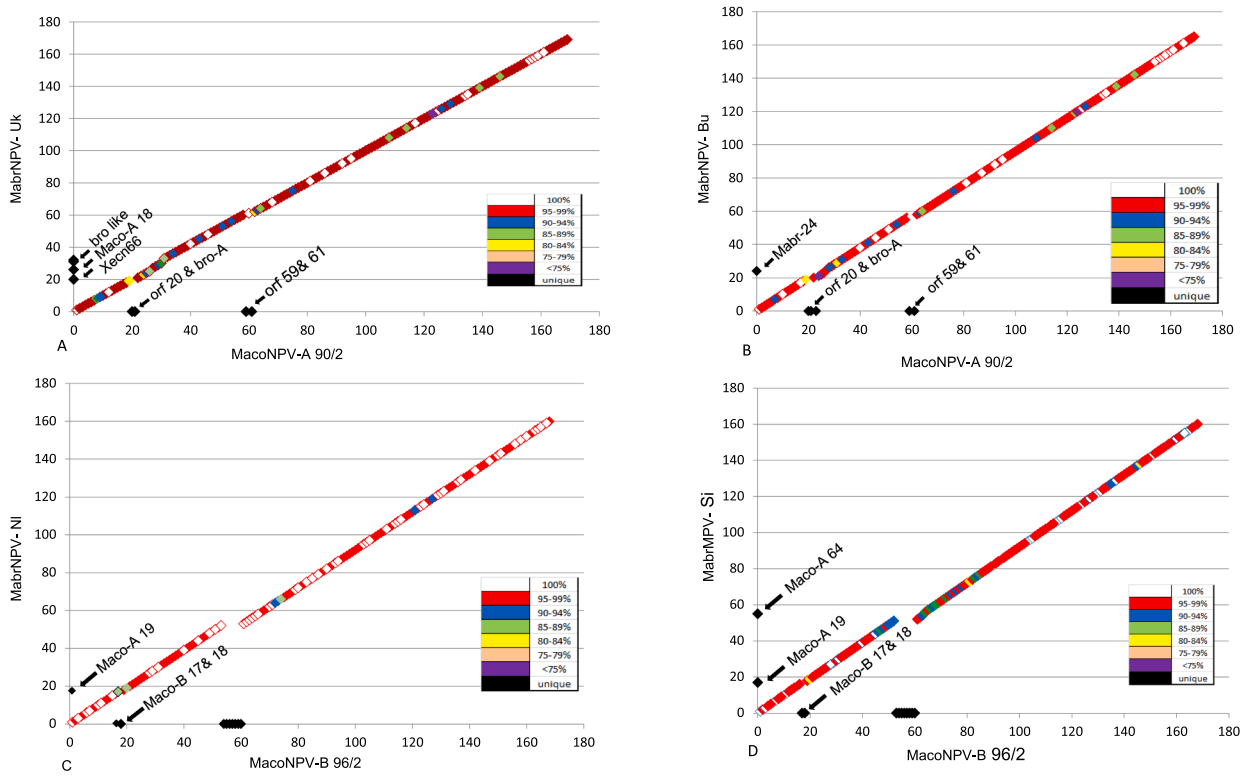

Fig. 3. Identity-GeneParityPlot of Eurasian alphabaculovirus genome ORF compared to those of MacoNPV-A and MacoNPV-B ORF annotation; including MabrNPV-Uk versus MacoNPV-A 90/2 (Fig. 3A), MabrNPV-Bu versus MacoNPV-A 90/2 (Fig. 3B), MabrNPV-Nl versus MacoNPV-B 96/2 (Fig. 3C), and MabrNPV-Si versus MacoNPV-B 96/2 (Fig. 3D).

the typical *bro* gene, have limited homology to each other, but both contain a baculovirus BRO family *N*-terminal domain (pfam02498). The vast majority of ORFs had > 95 % aa identity between the two virus isolates. The region of greatest variability between the two viruses and lowest predicted aa identity fell in the region between *hr1* and *bro-c* (Table 3). The MabrNPV-UK set of *bro*-like genes were among those with the lowest aa identify to MacoNPV-A 90/2 ORFs, these included BRO-B (80 %), BRO-C (79 %) and BRO-G (55 %) with the latter ORF being significantly truncated at the C-terminus, 141 aa versus 235 aa for MacoNPV-A 90/2 BRO-G. Other ORFs with less than 85 % aa identity to MacoNPV-A homologues included ORFs 19 (83 %), 26 (77 %) and 62 (83 %).

The Identity-Gene Parity Plot analysis for MabrNPV-Bu versus MacoNPV-A 90/2 was similar to that for MabrNPV-Uk except that MabrNPV-Bu did not contain a homolog of MacoNPV-A 90/2 ORF 23 and it only had one ORF (a homolog of MabrNPV-Uk ORF 26) that was not found in MacoNPV-A 90/2 (Fig. 3B). The region of greatest variability and lowest aa identity in ORF content between the two viruses, like MabrNPV-UK, fell in the region between *hr1* and *bro-c* (Table 3). The complement of *bro*-like genes were among those with the lowest aa identify to MacoNPV-A ORFs, these included BRO-B (81 %), BRO-C (83 %) and BRO-G (55 %) with the latter ORF being significantly truncated at the C-terminus, 140 aa versus 235 aa for MacoNPV-A 90/2 BRO-G. Other ORFs with less than 85 % ID to MacoNPV-A homologues included; MacoNPV A ORF 19 (84 %), 26 (77 %) and 62 (83 %), as was described above for MabrNPV-Uk. Both MabrNPV-Uk and -Bu had several ORFs with higher identity to the MacoNPV-B homologue than to MacoNPV-A homologues, these included homologues of MacoNPV-A 90/2 ORFs 26 (77 %), 62 (83 %) and 63 (92 %).

3.6. ORF analysis -MacoNPV-B -like isolates

MabrNPV-Nl and Si were found to be isolates of *Alphabaculovirus altermaconfiguratae*. These isolates, however, did not contain ORFs 17 and 18 when compared to the archetype isolate MacoNPV-B 96/2 (Fig. 3 C & D). In place of ORF17 and 18 there was a single ORF, which was 82 % identical to ORF 19 of the MacoNPV-A isolate MacoNPV-A 90/2. Interestingly, two previously sequenced Eurasian isolates, HearMNPV and MabrNPV-CHb, have orthologues of MacoNPV-B 96/2 ORF 18 but in different genomic locations, HearMNPV ORF 66 and MabrNPV-CHb1 ORF 134. Each of these putative proteins is substantially larger (591 aa) and has a putative transposase DNA binding motif (pfam 07282) at their C-terminus, similar to that found in MacoNPV-B ORF 18. MabrNPV-Si also has a second MacoNPV-A ORF, ORF55 which is a homologue of MacoNPV-A 90/2 ORF-64. No other MacoNPV-B virus from Eurasia nor MacoNPV-B 96/2 contain a homolog of MacoNPV-A 90/2 ORF-64. There is also a region in MabrNPV-Si spanning ORF46-85 in which the putative ORFs had higher aa identity to MacoNPV-A 90/2 ORFs than to any of the other MacoNPV-B ORF homologues.

As was noted for the MacoNPV-A Eurasian isolates, the region of greatest variation in ORF homology and content among the MacoNPV-B Eurasian isolates and MacoNPV-B 96/2 is the region between *hr1* and *bro-a* (Table 4).

3.7. bro gene variations

The North American MacoNPV-A isolates including MacoNPV-A 90/2, SK156, AB260 and others, contain 8 *bro* genes, the highest number among the MacoNPV-A and MacoNPV-B genomes sequenced (Fig. 4). This is due to the presence of the MacoNPV-A 90/2 *bro a* gene, positioned in a highly variable region between *hr1* and the *v-chi* gene. MacoNPV-A 90/2 *bro a* is found in some, but not all, North American MacoNPV-A isolates (Fig. 4). The North American MacoNPV-B viruses contained 7 *bro* genes, one more than the MacoNPV-B viruses from Eurasia which contain only 6 *bro* genes (Fig. 4). Interestingly the aa sequence encoded by positional homologues of the *bro* genes common

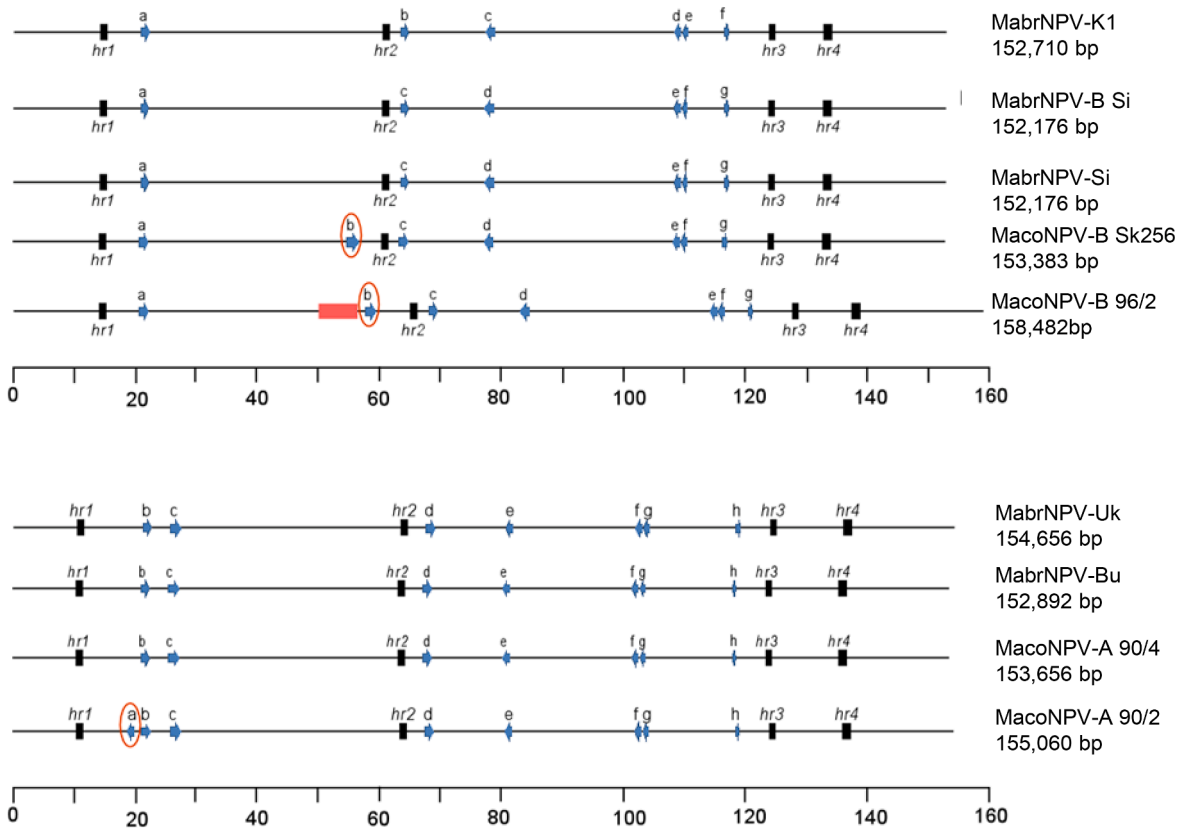

Fig. 4. Linearized genome maps displaying the relative genomic locations of the homologous repeat regions (*hr*) and *bro* genes on MacoNPV-A (lower panel) and MacoNPV-B viruses. The *hr* regions are shown as black boxes and the *bro* genes as blue arrows, designating direction of transcription. The b and a *bro* genes circled in red are not found in all genomes of the MacoNPV-B and MacoNPV-A isolates, respectively. The red box in MacoNPV-B 96/2 indicates the position of the XecnGV-like gene positional insertion. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

among the MacoNPV-A and MacoNPV-B like viruses grouped together in clades in an inferred phylogenetic tree generated from a maximum likelihood phylogenetic analysis of the BRO proteins (Fig. 5). Thus, our *bro* gene naming strategy linked positional information and phylogenetic relationships for the MacoNPV-A and MacoNPV-B viruses. For example, the MacoNPV-A 90/4 genome did not contain a MacoNPV-A 90/2 *bro-a* homologue and thus the first *bro* gene in the linear representation of the MacoNPV-A 90/4 genome and those of the Eurasian MacoNPV-A isolates was named *bro-b* (Fig. 4). The inferred phylogenetic tree indicated that BRO-A of MacoNPV-A separated as a unique taxonomic unit on a separate branch with high boot strap support (Fig. 5). The Eurasian MacoNPV-B like viruses did not have a homologue of the North American MacoNPV-B 96/2 BRO-B protein. It should be noted that for previously characterized genomes of Asian MabrNPV isolates the *bro* gene naming differs because they are named in the order of appearance on the genome without respect to homology considerations. As a result, the BRO-B homologues of MacoNPV-B North American isolates formed a separate phylogenetic branch with the Asian MacoNPV-A BRO-C representatives with high boot strap support (100 %) (Fig. 5). Interestingly, the remaining *bro* genes fell into two major clades of BRO proteins supported by high boot strap values, the first clade contained three taxonomic units including representatives of Maco-A BRO-E/Maco-B BRO-D/Mabr-K1 BRO-C, Maco-A BRO-F/Maco-B BRO-E/Mabr-K1 BRO-D, and Maco-A BRO-G/Maco-B BRO-F/Mabr-K1 BRO-E homologues, respectively (Fig. 5). The second major clade also contained three taxonomic units including representatives of Maco-A BRO-D/Maco-B BRO-C/Mabr-K1 BRO-B, Maco-A BRO-B/Maco-B BRO-A/Mabr-K1 BRO-A, and Maco-A BRO-H/Maco-B BRO-G/Mabr-K1 BRO-F homologues, respectively (Fig. 5). Within these two major clades there were a few anomalies with respect to the expected phylogenetic relationship groupings of MacoNPV-A versus MacoNPV-B homologues. For example, the BRO-F homologue of the MacoNPV-A isolates, MacoNPV-A Ls and MabrNPV-Uk did not group in a single clade with the other MacoNPV-A isolate BRO-F homologues (Fig. 5). Similarly, not all the MacoNPV-A BRO-B grouped together in a single taxonomic unit. Finally, the MabrNPV-Uk BRO-D homologue did not group together with the rest of the MacoNPV-A virus BRO-D genes but was more closely related to the MacoNPV-B BRO-C homologues (Fig. 5). As the *bro* genes were among the lower homology genes, ie., the most diverse genes, across the various MacoNPV-A and MacoNPV-B isolates it was not surprising that we observed some unexpected phylogenetic relations among this group of homologous genes. The diversity of *bro* genes has been known for a long time and have been associated with hot spots of recombination (Bideshi et al., 2003; Santos et al., 2018).

3.8. Oral infectivity bioassays

There were no statistically significant differences noted in the response of host insects to the various MacoNPV-A & -B like isolates, possibly due to variation between replicate assays (Table 5). However, there was a trend within individual replicate bioassays for MacoNPV-A like isolates to be less infectious for 2nd instar *T. ni* larvae than were the MacoNPV-B like isolates. The mortality at the high dose concentration (1.0x10^{7} OB/ml) with the MacoNPV-A like isolates ranged from 54 to 69 % while the MacoNPV-B like isolates produced 79–81 % mortality in 2nd instar *T. ni* larvae. The infectivity/mortality assays in 2nd instar *M. configurata* larvae showed no substantial difference in mortality response to any of the MacoNPV-A or –B like isolates (Table 5). In all cases the high dose inoculum produced significantly higher mortality than the low dose.

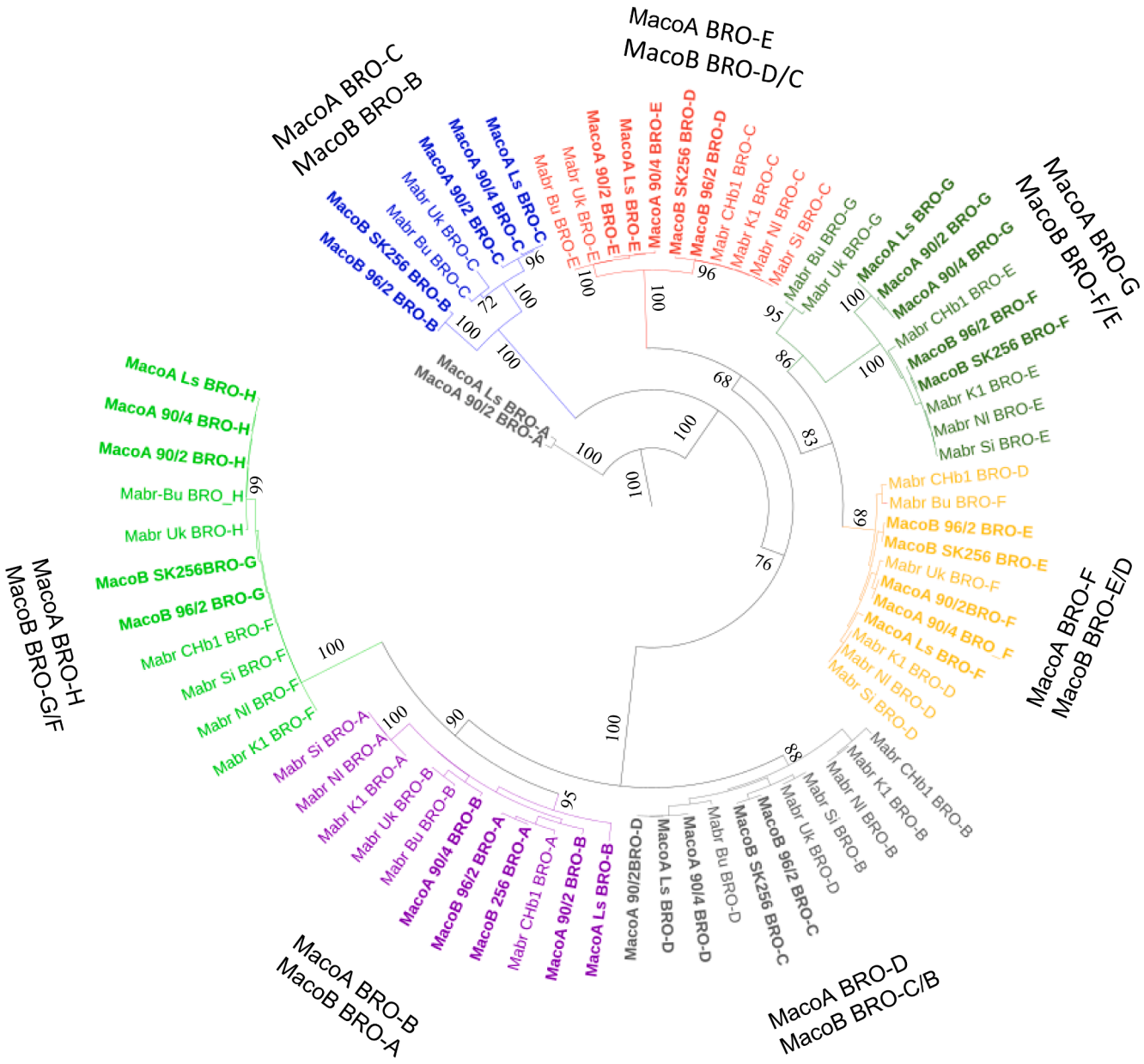

Fig. 5. Phylogenetic analysis of deduced amino acid sequence alignment of the BRO proteins from MacoNPV-A, MacoNPV-B and MabrNPV isolates. The maximum likelihood phylogram was inferred using the Neighbour Joining algorithm with the WAG amino acid substation model and 500 rounds of boot strap analysis. Numbers at nodes are bootstrap values greater than 50%. Each clade of homologous *bro* genes is highlighted in a different color.

4. Discussion

The alphabaculoviruses isolated to date from *M. configurata* populations in North America have been characterized as isolates of MacoNPV-A or MacoNPV-B based on restriction endonuclease profiles, whole genome sequencing (nucleotide sequence identity, gene content and synteny) and infectivity/virulence in a variety of potential host species (Li et al., 2001; Li et al., 2002; Li et al., 2005). The distinction of these alphabaculoviruses as belonging to two species was recently corroborated by the demonstration of K-2-P model phylogenetic distances of ~ 0.088 for single and concatenated *lef-8*, *lef-9* and *polh* gene sequences (Simón et al., 2017). These K-2-P values were consistently higher than the 0.05 value set as a demarcation of isolates constituting different baculovirus species (Jehle et al., 2006). Although historically there have been many descriptions of alphabaculoviruses isolated from European and Asian populations of *M. brassicae* (Aruga et al., 1960; Brown et al., 1981; Burgerjon et al., 1975; Possee & Kelly, 1988; Vlak & Gröner, 1980; Wiegers & Vlak, 1984), it is only relatively recently that complete genome sequences for some of these viruses have been published (Choi et al., 2013; Tang et al., 2012). The genome sequence of theses isolates, MabrNPV-K1 and HearMNPV, clearly demonstrated that they are very closely related to MacoNPV-B (Choi et al., 2013; Tang et al., 2012). We have demonstrated in the current study that MabrNPV-K1 and HearMNPV are strains of *Alphabculovirus altermaconfiguratae* and not a separate species. As a result we are proposing that the ICTV taxonomy statement be changed to reflect this fact.

Using multiplex PCR, REN analysis and whole genome sequencing approaches to characterize additional alphabaculovirus isolates, we have demonstrated that some of the previously described isolates from *Mamestra* populations in Eurasia constitute additional MacoNPV-B strains including the previously characterized MabrNPV-Nl, -De and -Si isolates (Vlak & Gröner, 1980; van Oers & Vlak, 2007). The identity of these isolates as MacoNPV-B species designation was based on the presence of a *hel-2* homologue (MacoNPV-B 96/2 orf 26) as well as their

Table 5
Virulence comparisons (% mortality at day 10 post infection) of MacoNPV-A and MacoNPV-B like isolates in *T. ni* or *M. configurata* 2nd instar droplet feeding bioassays.

	Virus Isolate	T. ni	M. configurata
High Dose	MacoA- 90/2	69.3 ± 4.9	63.9 ± 13.9
$1.0x10^7$ OB/ml	MacoA-Uk	57.1 ± 5.5	78.7 ± 9.0
	MacoA-Bu	54.1 ± 21.3	69.0 ± 10.6
	MacoB-96/2	78.9 ± 9.3	81.6 ± 10.2
	MacoB-Nl	80.0 ± 12.6	67.7 ± 15.3
	MacoB-De	81.1 ± 7.9	73.7 ± 5.7
		F5,17 = 1.06P = 0.43	F5,17 = 0.37P = 0.86
Low Dose	MacoA- 90/2	16.5 ± 6.1	16.7 ± 7.2
$1.0x10^5$ OB/ml	MacoA-Uk	18.1 ± 3.5	22.2 ± 5.5
	MacoA-Bu	11.1 ± 3.7	34.9 ± 8.8
	MacoB-96/2	30.9 ± 9.6	18.1 ± 8.4
	MacoB-Nl	36.7 ± 7.9	20.5 ± 7.1
	MacoB-De	28.3 ± 18.3	11.1 ± 7.4
		F5,17 = 1.05P = 0.43	F5,17 = 1.1P = 0.39

overall nucleotide sequence identity, gene synteny and *bro* gene content similarity to MacoNPV-B isolates from North America. In addition, we also demonstrated that MacoNPV-A strains, including MabrNPV-Uk and MabrNPV-Bu, also circulate in Eurasian *Mamestra* populations. The authenticity of the MacoNPV-A species designation for these isolates was based on the presence of a *lef-7* homologue (MacoNPV-A 90/2 orf 16) in both MabrNPV-Uk and –Bu as well as their overall nucleotide sequence identity, gene synteny and *bro* gene content similarity to MacoNPV-A isolates from North America. The characterization of these pools of alphabaculovirus isolates from North America and Eurasia, that are associated with *Mamestra* hosts, as belonging to two species, *Alphbaculovirus maconfiguratae* (MacoNPV-A) or *Alphabaculovirus altermaconfiguratae* (MacoNPV-B), is also supported by K-2-P distances estimates from partial *lef-8*, *lef-9* and *polh* gene sequences. We demonstrated that the K-2-P composition distances for MabrNPV-Uk and –Bu were 0.015 or lower when compared to MacoNPV-A 90/2 sequences for *lef-9, polh* and *lef-8/lef-9/polh* concatenated gene sequences and were 0.060 or higher when compared to MacoNPV-B 96/2 sequences, supporting the separation into two species. The *lef-8*K-2-P distances among the MacoNPV-A viruses were slightly higher (0.019–0.022) but still well below the 0.050 level suggested for species separation (Jehle et al., 2006). We also demonstrated that the K-2-P distance estimates for MabrNPV-Si and –Nl were 0.001 or lower when compared to MacoNPV-B 96/2 sequences for *lef-8*, *lef-9* and *polh* gene sequences and were 0.060 or higher when compared to MacoNPV-A 90/2 sequences again supporting the separation in two species MacoNPV-A and MacoNPV-B among both North American and Eurasian isolates.

The whole genome alignments and associated phylogenetic tree analysis for the North American and Eurasian isolates confirmed the division of MacoNPV-A and MacoNPV-B viruses into two major clades each made up of representatives from both geographic regions. Among the MacoNPV-B virus group, the MabrNPV-Si isolate was the most distinct with a genetic distance of 0.028 separating it from the other MacoNPV-B isolates (Fig. 2B). This genetic distance is also borne out in the homology/identity analysis comparing each MabrNPV-Si ORF aa sequence to that of MacoNPV-B 96/2 ORF aa sequence (Table 4). The MabrNPV-Si genes between *alk-exo* (orf47) and *38k* (orf79) encoded putative aa sequences that had higher homology to MacoNPV-A 90/2 ORFs than to MacoNPV-B 96/2 ORFs. This suggests that for this virus isolate there may have been a significant genetic recombination event between a MacoNPV-A and a MacoNPV-B virus, possibly during coinfection of the same host, an event which has been observed frequently among baculoviruses (eg. MabrNPV-Yu isolate Fig. 1; Erlandson et al., 2007).

Phylogenetic analysis of BRO amino acid sequences showed that MacoNPV-A and MacoNPV-B *bro* genes were not only positionally related within the species but the specific *bro* genes were also related evolutionarily (Fig. 5). However, there were some variations to the expected single clade relationship of BRO proteins within MacoNPV-A and MacoNPV B lineages. This was particularly apparent for MacoNPV-A like virus MabrNPV-Uk with respect to BRO-B and –D and MabrNPV-Bu BRO-B and –F. However, North American MacoNPV-A virus isolates MacoNPV-A 90/4 and –Ls BRO-B proteins showed divergence from MacoNPV-A 90/2 BRO-B.

We previously demonstrated that the host range of MacoNPV-B is potentially broader than that for MacoNPV-A using dose response comparisons in 3rd instar diet plug feeding bioassays with a limited selection of three noctuid species; *M. configurata*, *T. ni* and *S. exigua* (Li, L. *et al.,* 2002). The observed differences in LD_{50} values were particularly significant in the case of *S. exigua* hosts (MacoNPV-A > $1.0x10^4$ OB/larva versus $1.2x10^3$ OB/larva for MacoNPV-B), while there was a trend to higher virulence for MacoNPV-B in *T. ni* larvae versus MacoNPV-A but it was not statistically significant. In the current study we also found that MacoNPV-B viruses including MacoNPV-B 96/2, MabrNPV-Nl and MabrNPV-De trended to being somewhat more infectious (28.3–36.7 % mortality at the low $1.0x10^5$ OB/ml dose) than MacoNPV-A viruses (11.1–18.1 % mortality at the low $1.0x10^5$ OB/ml dose) for 2nd instar *T. ni* larvae in a two-dose droplet feeding bioassay (Table 5). However, as was the case with our previous investigations of virus infectivity in bioassays with 2nd and 3rd instar *M. configurata* larvae, these differences were not significantly different (see Table 5; Li, L. *et al.*, 2002). Few studies have directly compared the infectivity of North American and Eurasian MacoNPV-A and MacoNPV-B like virus isolates against *Mamestra* hosts. We previously undertook droplet feeding bioassays in neonate *M. configurata* larvae and found no significant difference between LD_{50} values for MacoNPV-A isolate 86/1 or MabrNPV-Nl and Nl-82/1 isolates; however, MabrNPV-De produced significantly higher LD_{50} value estimates (Erlandson, 1990). In the current study, infectivity/mortality assays in 2nd instar *M. configurata* larvae showed no significant difference in mortality response to the MacoNPV-A or –B like isolates. Comparison of the earlier study with the bioassay results of this study (Table 5) is difficult because this study was conducted with a different larval instar stage and not with a full dose–response assay. Recently, a battery of 8 noctuid host species were tested in 2nd instar droplet feeding bioassays using MacoNPV-A 90/2, MacoNPV-B 96/2, MabrNPV Mamestrin™ isolates and a previously uncharacterized alphabaculovirus from *Lacanobia oleracea* (Simón et al., 2017). MacoNPV-B was found to be more pathogenic than MacoNPV-A for several species including *M. brassicae, T. ni, S. exigua,* and *Chrysodeixis chalcites*. Additionally, both MacoNPV-A and -B viruses were more pathogenic to *M. brassicae* than to the homologous host, *M. configurata*. Mortality data from this study confirmed that MacoNPV-B isolates trended to higher mortality rates in 2nd instar *T. ni* than did MacoNPV-A isolates.

As indicated above, all of the MacoNPV-A type viruses sequenced to date contain a *lef-7* homologue (MacoNPV-A 90/2 orf 16) and always adjacent to *hr-1*. Homologues of *lef-7* have been found in all Group I alphabaculoviruses and a select few Group II alphabaculoviruses including Spodoptera exigua MNPV, Spodoptera frugiperda MNPV, MacoNPV-A and recently in Mythimna unipuncta nucleopolyhedrovirus #7 (MyunNPV#7) (Harrison et al., 2018). LEF-7 is an F-box protein that has been implicated in modifying the host DNA damage response machinery and thereby enhancing virus DNA replication (Mitchell et al., 2013). Interestingly, MacoNPV-B isolates do not contain a *lef-7* homologue. However, MacoNPV-B isolates contain a homologue of a gene found predominantly in betabaculoviruses, namely *helicase-2* (MacoNPV-B 96/2 orf 13), which has not been identified in any of the MacoNPV-A isolate genomes. Homologues of *helicase-2* are found in many of the betabaculovirus genomes and in a few Group II alphabaculoviruses. The Lymantria dispar MNPV (LdMNPV) *helicase-2* was shown to have no effect on LdMNPV DNA replication in transient assays and could not replace the function of *helicase/p143* (Pearson and

Rohrmann, 1998). LdMNPV *helicase-2* has some homology to a yeast helicase that functions in repair and recombination of mitochondrial DNA (Rohrmann, 2013). It is not known if the differential presence of the *lef-7* and *helicase-2* in MacoNPV-A and MacoNPV-B isolates, respectively has any relationship with the host range and virulence of these two viruses.

In summary, using whole genome sequencing, multiplex PCR and REN analysis we demonstrated that previously isolated NPVs from *Mamestra* populations in Eurasia included isolates of both *Alphabaculovirus maconfiguratae*, and *Alphabaculovirus altermaconfiguratae*. The MabrNPV-Bu and –Uk isolates contained an *lef-7* homologue and had ~ 96 % nt identity to MacoNPV-A 90/2 at the whole genome level. Similarly, the MabrNPV-Si, -De and –Nl had 96.6, 96.6 and 98.5 % identity to MacoNPV-B 96/2 at the whole genome level, respectively. The gene content, synteny and K-2-P *lef-8*, *lef-9* and *polh* analysis also confirmed the presence of both MacoNPV-A and MacoNPV-B isolates in Eurasia. Thus, both these alphabaculovirus species have wide Holarctic distributions in *Mamestra* host species; and while both species have wide host ranges, MacoNPV-B showed higher levels of infectivity for the widest range of host species. However, we did not find definitive evidence for spatial gradients of genotypes over wide geographic distances for either MacoNPV-A or MacoNPV-B isolates.

CRediT authorship contribution statement

Martin Erlandson: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Doug Baldwin:** Data curation, Formal analysis, Investigation, Methodology, Validation. **Just M. Vlak:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Writing – original draft. **David Theilmann:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study was funded in part by an Agriculture and Agri-Food Canada, Canadian Crop Genomics Initiative (J001033) grant (MAE & DAT). We thank Cathy Coutu for assistance with bioinformatics and Ruwandi Andrahennadi for providing insects for bioassays.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2024.108063.

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